Identification of the Specific Oligosaccharide Sites Recognized by Type 1 Fimbriae from *Escherichia coli* on Nonspecific Cross-reacting Antigen, a CD66 Cluster Granulocyte Glycoprotein*

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Non specific cross-reacting antigen (NCA), a CD66 cluster antigen, is a well characterized glycoprotein on granulocytes, macrophages, and lung epithelium. Structural studies at the protein and genomic levels have revealed that NCA is a member of the immunoglobulin (Ig) supergene family and contains a domain structure similar to Ig with an amino-terminal variable-like domain followed by disulfide loop-containing constant-like domains. Previous work by this laboratory and others has demonstrated that NCA is a receptor for binding of bacteria expressing type 1 fimbriae (pili). This binding is mediated by interaction between lectins on the bacteria fimbriae and carbohydrate chains on NCA. In the present work we further characterize the specificity for bacterial binding by NCA using endoglycosidases and site-directed mutagenesis.

Results of these studies demonstrate that *Escherichia coli* expressing type 1 fimbriae binds to high mannose oligosaccharide structures on NCA and that the functionally relevant sites are located in the variable-like domain of NCA.

Granulocytes are vital cells in the immune response to bacterial infections. Activation of granulocytes by bacteria or other stimuli leads to a series of cellular responses, including aggregation, stimulation of the respiratory burst, degranulation, increased phospholipid turnover, depolarization of the plasma membrane, and phagocytosis of the bacterium (1-6). These responses are mediated by the binding of a variety of ligands to specific receptors, such as the binding of opsonized bacteria to the Fc or CR3 receptors. In addition to the role of the Fc and CR3 receptors, phagocytic cells bind bacteria by a carbohydrate-specific interaction between membrane glycoproteins and lectins on bacterial fimbriae. This form of binding and response, termed lectinophagocytosis, does not require opsonization of the bacteria with serum factors and is considered the first line of defense against bacterial infections (7).

Numerous studies have demonstrated the involvement of lectin-mediated binding of bacteria to phagocytes during immune response to systemic infections (reviewed in Ref. 7). Binding of *Escherichia coli* expressing type 1 fimbriae to human leukocytes in the absence of serum factors increased phagocytosis (8), chemiluminescence (9-11), protein iodination (12), degranulation (13), and activation of protein kinase C (14). Studies with latex particles coated with isolated type 1 fimbriae demonstrated that the fimbriae per se are capable of mediating activation of granulocytes (9).

Oligosaccharide sequences identified as receptors for fimbriae include Manα1-3Man for type 1 fimbriae, NeuAcα2-3Gal for S-fimbriae, and Galα1-4Gal for P-fimbriae (15). The mannose-specific type 1 fimbriae from *E. coli* are the most thoroughly characterized. Type 1 fimbriae are composed of 17-kDa subunits assembled into a right-handed helix (16). The mannose binding site is on a minor subunit (M, 29,000) located at the tips and at long intervals along the length of the fimbriae (17-19). Regulation of expression of type 1 fimbriae occurs at the transcriptional level, allowing bacteria to switch between the Fim<sup>+</sup> and Fim<sup>-</sup> phenotypes (20). Various carbohydrates and their conjugates have been tested to define the specificity of the type 1 fimbriae lectin binding site (15). The current model proposes that the binding pocket is specific for a high mannose saccharide and contains a hydrophobic binding region in close proximity to the saccharide binding site (15). Preference for hybrid oligosaccharides is suggested by some studies (15). The absence of structural data on type 1 fimbrine-binding glycoproteins has prevented detailed analysis of the ligand structure.

Although many studies have addressed the role of bacterial fimbriae in mediating adhesion to, and activation of phagocytes, little is known about the leukocyte glycoproteins that are recognized by the fimbriae. Sharon and co-workers (21) have identified membrane glycoproteins from human granulocytes that act as receptors for type 1 fimbriae. These receptors have apparent molecular mass values of 45, 70-80, 100, and 150 kDa. Later studies by this group have identified the 150-kDa glycoprotein as the granulocyte complement receptor CD11b/CD18 (22). No evidence is available concerning the identities of the other glycoproteins identified by Sharon (21).

Recently our group (23, and that of Wagener (24), have demonstrated that members of the nonspecific cross-reacting antigen (NCA)<sup>1</sup> family are receptors for type 1 fimbriated bacteria. The NCA family is composed of highly glycosylated proteins that are related immunologically and structurally to carcinoembryonic antigen, a relationship from which the

<sup>1</sup>The abbreviations used are: NCA, nonspecific cross-reacting antigen; CEA, carcinoembryonic antigen; BGP I, biliary glycoprotein I; GPl, glycosylphosphatidylinositol; TOF-MS, time-of-flight mass spectrometry; endo H, endoglycosidase H; FCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; TFMSA, trifluoromethanesulfonic acid; CHO, Chinese hamster ovary.
name is derived. NCA is found on lung epithelium, macrophages, and granulocytes, and can be induced on colonic adenocarcinoma (25-28). Structural studies on the isolated proteins (29) and from cDNA sequencing (30-32) have revealed much concerning the identities of the various NCA glycoproteins. The NCA glycoprotein family is a large immunoglobulin supergene family and have a domain structure similar to immunoglobulins with an NH$_2$-terminal variable-like domain followed by two disulfide loop-containing constant-like domains. NCA-50 (Mf, 50,000) and NCA-90 (M, 90,000) (formerly called TEX-75) were found to be identical with respect to sequence and placement of glycosylation sites despite differing by 40,000 in apparent mass (29). These glycoproteins each contain three N-linked glycosylation sites in the variable-like NH$_2$-terminal domain and nine sites in the constant-like domains (29). NCA-95 has a unique sequence but is homologous to NCA-50/90 (30-32). Protein sequence data on granulocyte NCA-160 and immunological studies by Drzeniek et al. (46) have demonstrated that this form of NCA is identical to biliary glycoprotein I (BGP I) (33). NCA-50, NCA-90, and NCA-95 are anchored to the membrane through a glycosylphosphatidylinositol anchor, while NCA-160 is believed to be an integral membrane protein with a transmembrane and cytoplasmic domain.

The expression on phagocytic cells and the structural similarity with immunoglobulins suggest a function for NCA in immune recognition. This proposal is strengthened by the work of Kantor et al. (34), who demonstrated a 6-10-fold elevation of NCA mRNA expression in colon tumor cell lines following treatment with gamma interferon. These observations led our group and that of Wagener to examine the bacterial binding properties of NCA-50 (23, 24). These studies established that NCA-50 binds to bacteria expressing type 1 fimbriae and that the binding is inhibited by alpha-d-mannose or derivatives of alpha-d-mannose (23, 24). Wagener and co-workers (24) also showed the bacterial binding properties of CEA and BGP. We have in addition demonstrated the increased surface expression of NCA upon activation of granulocytes (23), a finding consistent with a role for NCA in bacterial binding. These studies were later confirmed by Kuroki et al. (35). In the present report we examine the glycosylation sites responsible for bacterial binding. Here we focus on the role on the three sites in the variable-like NH$_2$-terminal domain of NCA-50.

**MATERIALS AND METHODS**

**Biotinilation of Type 1 Fimbriae**—Type 1 fimbriae were isolated from *E. coli* CSH50 (19) according to the method of Eshdat et al. (36). The purity of the fimbriae was examined by electron microscopy, amino acid analysis, and immunoreactivity with anti-type 1 polyclonal antibodies which were generously provided by S. N. Abraham (Washington University, St. Louis, MO) (data not shown). Type 1 fimbriae (5 mg/ml) were washed twice in 50 mM bicarbonate buffer, pH 8.5, and biotinylated with 5-bromo-4-chloro-3-indolyl phosphate (50 mg/ml) and nitroblue tetrazolium (100 mg/ml). The solutions were dried, resuspended in deionized water, and analyzed with a Bio-LC system (Dionex Corp.) equipped with an amperometric detector.

**Plasmids, Enzymes, and Antibodies**—The vectors pHPAPr-1-neo (38) and pHsAPr-1-neo/NCA containing the NCA cDNA (32) were provided by Dr. J. L. Hefta (Beckman Research Institute, City of Hope, Duarte, CA). The NCA cDNA was converted from the GPI-anchored form to the secreted form by introducing an amino-terminal restriction enzyme site (SalI) and a HindIII site at the 3'-end of the NCA cDNA (32). All plasmids were purified on a preparative scale (36). The restriction enzymes were purchased from Boehringer Mannheim, the reverse transcriptase (AMV-Super RT) from LifeScience, and the GeneAmp Kit from Perkin Elmer Cetus.

**Site-Directed Mutagenesis of the NCA Glycosylation Mutants**—Site-directed mutagenesis of the first three glycosylation sites of NCA in the NH$_2$-terminal domain (29) was carried out according to the PCR-mediated mutagenesis strategy described by Nelson and Long (40). For these studies, a SalI restriction enzyme site was introduced 5' and a HindIII site 3' of the start and stop codons, respectively. Site-directed mutagenesis of the glycosylation sites (A1,2-s, and A1,2,3-s) was performed using the plasmid pHsAPr-1-neo/NCA-s (39). The restriction enzymes were purchased from Boehringer Mannheim, the reverse transcriptase (AMV-Super RT) from LifeScience, and the GeneAmp Kit from Perkin Elmer Cetus.

**Transfection of HeLa Cells**—Stable transfechypenotypes of the NCA mutants were generated by lipofection of HeLa cells. HeLa cells (5 x 10$^5$) seeded into a tissue culture dish 24 h prior to the lipofection) were grown at 37°C with 5% CO$_2$ in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 10 mM Hepes buffer (100 units/ml), and streptomycin and amphotericin B (100 units/ml). Plasmid DNA (30 μg) and Lipofectin™ (10 μg) were mixed in 4 ml of Opti-MEM (Life Technologies, Inc.) medium, and the cells incubated

for 8 h. The Lipofectin/plasmid mixture was removed, and the cells selected in supplemented Dulbecco’s modified Eagle’s medium containing 1 mg/ml G-418 for 7 days. Resistant colonies were cloned with cloning rings and the supernatant tested for secreted NCA by an indirect enzyme-linked immunosorbent assay (ELISA). For the ELISA, a NCA-reactive polyclonal rabbit antibody (DAKO, Carpinteria, CA) was coated on a microtiter plate (Immunolon 2, Dynatech) and, after blocking of free binding sites with 3% (w/v) BSA, the plate was incubated with the supernatant from growing G-418-resistant clones. The anti-NCA-reactive monoclonal antibody T84.1E3 was allowed to react with bound NCA followed by the incubation with an alkaline phosphatase-labeled goat-anti mouse antibody and the addition of the substrate p-nitrophenyl phosphate (1 mg/ml). Bound NCA was detected by the color reaction at 405 nm in a Multiscan photometer. The highest producer was identified by the determination of the specific productivity (µg of protein/1 × 10^6 cells in 24 h) in comparison with a known amount of NCA-50 using the indirect ELISA.

**Purification and Time-of-Flight Mass Spectrometry (TOF-MS) of the Secreted NCA Glycosylation Mutants—**The secreted forms of NCA-50 wild-type and the five glycosylation mutants were purified from the supernatant over a T84.1E3 affinity column. The affinity column was prepared as described by the manufacturer (Sterogene, Arcadia, CA) and equilibrated with phosphate-buffered saline (PBS). The supernatant of NCA-secreting HeLa cell transformants was applied directly and the column subsequently washed with PBS, BBS (0.1 M H₂BO₃, 0.025 M Na₂BO₂, 1 M NaCl, 0.1% (v/v) Tween 20, pH 8.3), and again with PBS before eluting the bound NCA with 0.1 M glycine (pH 2.5). The NCA-containing fraction was dialyzed against deionized water, lyophilized, and analyzed for purity by amino-terminal sequence analysis and SDS-PAGE. The molecular masses of the various secreted NCA glycosylation mutants were determined with TOF-MS (LAMS-50KS, Shimadzu). Approximately 20 pmol of the glycoprotein were dissolved in 2 µl of 30% (v/v) acetonitrile containing 0.1% (v/v) trifluoroacetic acid and 2 µl of saturated sinapinic acid in 30% acetonitrile containing 0.1% trifluoroacetic acid, the sample applied to a copper stage, dried, and ionized with a neodymium YAG laser at 355 nm.

**Binding Assay with the NCA Glycosylation Mutants and Type 1 Fimbriae—**The interaction between type 1 fimbriae and the various NCA glycosylation mutants was quantified in a solid-phase assay. For this, a microtiter plate (Immunolon 2, Dynatech) was coated with type 1 fimbriae (20 µg/ml), free binding sites blocked with 3% (w/v) BSA, and the NCA wild-type or glycosylation site mutants (100 pmol/well) added to the wells. Detection of the bound NCA was accomplished by addition of a NCA-reactive polyclonal antibody (DAKO), followed by incubation with alkaline phosphatase-labeled goat-anti rabbit antibodies, and color development with p-nitrophenyl phosphate (1 mg/ml). See Results section for additional explanation of this assay.

**RESULTS**

**Lectin Blots with Biotinylated Type 1 Fimbriae—**To identify the glycosylation sites on NCA-50 that bind to type 1 fimbriae, it was necessary to develop an expression system that would glycosylate NCA-50 correctly. HeLa cells were determined to be the correct cell line, as demonstrated by the following. A secreted form of NCA cDNA (see "Materials and Methods") was transfected into HeLa cells, and the secreted NCA was purified from the supernatant with an immunoaffinity column. The HeLa NCA along with other control glycoproteins were electrophoresed on two identical SDS-polyacrylamide gels and used in a lectin blot assay to demonstrate fimbriae binding. One of the gels was stained with Coomassie Blue to confirm that the same amount of each glycoprotein was applied (Fig. 1B). The glycoproteins of the other gel were transferred to nitrocellulose, the membrane incubated with biotinylated type 1 fimbriae, and bound fimbriae detected with alkaline phosphatase-labeled streptavidin (Fig. 1A). The lectin blot in Fig. 1A showed that biotinylated type 1 fimbriae specifically bound NCA-50 from colonic carcinoma (lane 2) but did not bind to the control glycoproteins, ovalbumin (lane 4, with high mannose type sugars) or fetuin (lane 5, which contains complex type sugars). A slight amount of binding was evident to NCA-90 (lane 1, a higher glycosylated glycoform of NCA-50), which is consistent with our previous binding studies (23). NCA secreted by HeLa cells (lane 3) was also bound by type 1 fimbriae, although to a lesser extent than tumor NCA-50. In contrast, there was no binding of type 1 fimbriae to NCA expressed by CHO-K1 or COS-1 cells (data not shown). A slight difference in apparent molecular weight was observed between NCA expressed in HeLa cells and that purified from tumor (Fig. 1B, lanes 2 and 3) with HeLa NCA having a slightly higher weight. These forms of NCA were further analyzed by TOF-MS to ascertain more accurate masses. Results from these analyses showed that HeLa NCA had a mass of 51,900 daltons in comparison to 46,052 dalton for NCA-50 (data not shown). The discrepancy in molecular mass is likely due to differences in glycosylation, such as microheterogeneity of the sugar structures. Nevertheless, the HeLa NCA (lane 3) showed type 1 fimbriae binding characteristics of tumor NCA-50 (lane 2), suggesting that HeLa NCA is glycosylated similarly, and that the expression of NCA-50 in HeLa cells can be used as a model system for studies with glycosylation mutants.

The lectin blot in Fig. 2 was carried out in order to examine whether type 1 fimbriae from *E. coli* CSH50 mainly recognize the mannose residues of the oligomannose type sugars of NCA-50. The same amounts of NCA-50, endo H-treated NCA-50 (lacking the high mannose type sugars only), deglycosylated NCA-50, and reduced and alkylated NCA-50 were separated in two identical SDS-gels. The protein samples were transferred to nitrocellulose and detected with anti-NCA monoclonal antibody T84.1E3 (Fig. 2A) or with biotinylated type 1 fimbriae (Fig. 2B). To quantitatively determine the binding of type 1 fimbriae to the differently treated NCA-50s, both blots were scanned with a densitometer (Biovision Densitometer, Applied Image). Results from the densitometer indicated that monoclonal antibody T84.1E3 bound equally to reduced and alkylated NCA-50 (Fig. 2A, lane 4), endo H-treated (lane 2), and deglycosylated NCA-50 (lane 3). In contrast, the interaction between the carbohydrate component of NCA-50 and the lectin on type 1 fimbriae is strongly influenced by these modifications (Fig. 2B). Fig. 2B shows that the type 1 fimbriae interacted strongest with reduced and alkylated NCA-50 (lane 4), less with untreated NCA-50 (lane 1), and weakest with endo H-treated NCA-50 (lane 2). It is likely that the denaturation of NCA-50 (lane 4) revealed...
additional binding sites for type 1 fimbriae. Deglycosylated NCA-50 (lane 3) was not recognized by type 1 fimbriae. In order to compare the effect of endo H treatment on the binding of type 1 fimbriae, it is important to note that the NCA-50 used in this analysis was reduced and alkylated prior to the endo H treatment. Results from the densitometric scan of the endo H (lane 2) and the reduced and alkylated (lane 4) indicated that endo H treatment reduced binding by 85%. It is possible that the slight binding observed following endo H treatment may involve hybrid types of sugars that would not be cleaved with endo H, or may be due to incomplete removal of oligomannose structures by the enzyme. These results, however, indicate that type 1 fimbriae mainly recognize the high mannose type oligosaccharides present on NCA-50.

Carbohydrate Compositional Analysis of NCA-50 and NCA Secreted by HeLa Cells—The carbohydrate composition of the HeLa secreted and tumor-isolated NCA-50s were examined to establish possible differences in patterns of glycosylation. The most significant difference between the carbohydrate analysis of the two NCAs is the higher amount of galactose in NCA secreted by HeLa cells, indicating more complex and/or hybrid types of sugars than in NCA-50 (Table I). This carbohydrate analysis is in accordance with the higher molecular weight of NCA secreted by HeLa cells versus tumor NCA-50 (Fig. 1A), since in mammalian cells the average weight of a complex type sugar is generally higher than that of a high mannose type sugar.

Mutagenesis of the NCA-50 cDNA—In order to determine which of the carbohydrate chains of NCA-50 is (are) responsible for the interaction with type 1 fimbriae, site-directed mutagenesis of the NCA-50 cDNA was performed to eliminate the first three glycosylation sites in the NH2-terminal domain of NCA-50. This domain was chosen for study in consideration of the extent of structural homology between it and the variable region of immunoglobulins (43) and in consideration of previous data that suggest a biological function for this domain (44). Mutagenesis of the NCA-50 cDNA produced five plasmids containing NCA glycosylation site mutations: pH3/NCA Δ1, pH3/NCA Δ2, and pH3/NCA Δ3 with individual sites deleted, pH3/NCA Δ1,2 with the first two sites deleted, and pH3/NCA Δ1,2,3 with all three of the NH2-terminal glycosylation sites deleted (see Fig. 3). The full length of the NCA-50 cDNA was sequenced in all five plasmids to verify the introduced mutations and to rule out mismatches introduced by Taq polymerase. Fig. 4 compares the DNA sequences of these five mutagenized plasmids with the wild-type plasmids in the area of the first three mutagenized asparagine codons. All PCR-mediated mutations were determined to be correct. In addition to the desired mutations, the Taq polymerase introduced silent mutations (indicated with a dot) in the cases of pH3/NCA Δ2 (Fig. 4, lane 3), pH3/ NCA Δ3 (lane 4), and pH3/NCA Δ1,2,3 (lane 6). Since these mutations were silent, the plasmids were used for the subsequent transfection experiments. In order to facilitate purification of the NCA mutant glycoproteins, the mutants were converted from their membrane-bound, GPI-anchored to the secreted form as described under “Materials and Methods.”

Transfection and Expression of NCA in HeLa Cells—The plasmids pH3APr-1-neo carrying the wild-type NCA-50 cDNA and each of the five NCA glycosylation mutants were integrated into the HeLa cell genome via liposome-mediated transfection. The respective NCA glycoproteins were expressed and secreted. The highest producing transformants were identified by indirect ELISA and used for the production of secreted NCA for subsequent studies. For each of the five glycosylation mutants and the wild-type NCA, one clone with a specific productivity (μg/1 × 106 cells in 24 h) of at least 1.5 μg was found. The six transformants with the highest specific productivity (1.5 μg for NCA-s, 1.5 μg for NCA Δ1-s, 1.5 μg for NCA Δ2-s, 3 μg for NCA Δ3-s, 1.5 μg for NCA Δ1, 2-s, and 7.8 μg for NCA Δ1, 2,3-s) were used for large scale production of the secreted mutants and wild-type NCAs.

Purification and Molecular Weight Determination of the NCA Glycosylation Mutants—The secreted wild-type and mutant NCA glycoproteins were isolated from approximately 500 ml of supernatant. After affinity purification with the NCA-reactive monoclonal antibody T84.1E3, 160–600 μg of each NCA was isolated (data not shown). No contaminating proteins were identified by amino-terminal sequence analysis or following SDS-PAGE (data not shown). The molecular masses of these purified NCA glycoproteins were determined by TOF-MS with representative spectra shown in Fig. 5. The molecular weights are: 51,900 for NCA-s, 50,000 for NCA Δ1-s, 50,600 for NCA Δ2-s, and 49,000 for NCA Δ3-s. In comparison with wild-type NCA, the carbohydrate components of the glycosylation mutants in the NH2-terminal domain of HeLa NCA showed a mass of 1,900 for the first, 1,300 for the second, and 2,900 for the third glycosylation site (Fig. 5). The molecular weights of the glycoproteins NCA Δ1,2-s and NCA Δ1,2,3-s were 48,600 and 45,200, corresponding to the loss of two and three oligosaccharide chains, respectively (spectra not shown).

Binding Assay of Biotinylated Type 1 Fimbriae to NCA Glycosylation Mutants—To ascertain the possible involve-
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Fig. 3. Nomenclature and amino acid exchanges for the five NCA glycosylation mutants in comparison to the wild-type NCA sequence (32). The sequences of the mutagenesis primers Δ1-Δ3 are listed. The asparagine residues (Asn) mutated to aspartic acid (Asp) or threonine (Thr) are in boldface type.

Fig. 4. Autoradiogram of the NCA cDNA sequences of pH3/NCA, wild-type (lane 1); pH3/NCA Δ1 (lane 2); pH3/NCA Δ2 (lane 3); pH3/NCA Δ3 (lane 4); pH3/NCA Δ1,2 (lane 5); and pH3/NCA Δ1,2,3 (lane 6). The plasmid DNA was purified over a Qiagen column, denatured, and sequenced using the T7 sequencing kit (U. S. Biochemical Corp.) according to the manufacturer’s directions. The PCR-mediated mutations of the glycosylation sites are indicated with an arrow and the silent mutations with a dot.

Fig. 5. Time-of-flight mass spectra of the NCA glycosylation mutants NCA Δ1-s, NCA Δ2-s, and NCA Δ3-s in comparison to the wild-type (wt) NCA.

Fig. 6. Reactivity of the six secreted and affinity-purified HeLa glycoproteins NCA-s (wild-type), NCA Δ1-s, NCA Δ2-s, NCA Δ3-s, NCA Δ1,2-s, and NCA Δ1,2,3-s with type 1 fimbriae in the solid phase assay. The reactivity of the HeLa glycoproteins with type 1 fimbriae is given as percentage and was measured in the linear range. The average values are taken from two independent experiments with three repeats each. The concentrations of fimbriae and the NCA species were determined as described under “Results” and were equal for all binding experiments.
In this study, we demonstrate the specific binding of type 1 fimbriae from *E. coli* CSH50 to NCA-50 and NCA-50 secreted from HeLa cells. NCA-50 is a well characterized glycoprotein found primarily on granulocytes and lung epithelium but also present on colonic adenocarcinomas. The interaction between NCA-50 and the bacterial fimbriae lectin is carbohydrate-specific with type 1 fimbriae binding to high mannose type sugars of the NCA-50 carbohydrate component. Sequential elimination of the first three of 12 glycosylation sites of the NCA-50 cDNA, subsequent expression in HeLa cells, and binding assays with type 1 fimbriae revealed that the first two oligosaccharide side chains significantly contribute to the binding of type 1 fimbriae whereas the third, and most likely the other nine, side chains do not interact with type 1 fimbriae.

The carbohydrate structures that are mainly responsible for the recognition of NCA-50 by type 1 fimbriae are the high mannose type sugars since there was a decrease of 85% in binding to NCA-50 after treatment with endoglycosidase H (Fig. 2). This result is in accordance with studies by Sharon and co-workers (15) showing that the binding pocket of type 1 fimbriae from *E. coli* is best fitted for the trisaccharide α-D-Man(1–3)-β-D-Man(1–4)-α-GlcNAc, a carbohydrate structure found in high mannose type sugars. The presence of high mannose type structures alone is, however, insufficient for the specific binding to type 1 fimbriae since ovalbumin and other glycoproteins with solely high mannose type sugars are not recognized by type 1 fimbriae (Fig. 1A and Sauter et al. (23)). Sharon and co-workers (45) suggest that the specificity for type 1 fimbriae is influenced by a hydrophobic component in close proximity to the trisaccharide Man(α1–3)-Man(α1–4)-GlcNAc. In this context one can speculate that the specific type 1 fimbriae for NCA is formed by a high mannose type structure in concert with hydrophobic amino acids.

In order to determine which of the 12 glycosylation sites of NCA were responsible for the interaction with type 1 fimbria and to further elucidate this carbohydrate structure, a model system was established by expressing NCA-50 and glycosylation mutants of NCA-50 in HeLa cells. HeLa cells were chosen as expression systems for the following reasons. 1) Messenger RNA transcripts of NCA were responsible for the interaction with type 1 fimbriae since there was a decrease of 85% in the molecular weights of HeLa NCA-50 and tissue NCA-50, with the former weighing approximately 50,653. This result is in accordance with studies by Sharon and co-workers (23) that none of the additional nine glycosylation sites in the lectin binding sites. In this respect, the cloned and expressed versions of NCA-50 in HeLa cells should form the basis from which the precise lectin specificity of *E. coli* type 1 fimbriae can be determined.

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