INFECTION AND IMMUNITY, Apr. 1987, p. 968–973
0019-9567/87/040968-06$02.00/0

Vol. 55, No. 4

Membrane Glycoproteins of Human Polymorphonuclear Leukocytes That Act as Receptors for Mannose-Specific *Escherichia coli*

MORELLA RODRIGUEZ-ORTEGA,[1]* ITZHAK OFEK,[2] AND NATHAN SHARON[1]

Department of Biophysics, The Weizmann Institute of Science, Rehovoth 76100,[1] and Department of Human Microbiology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978,[2] Israel

Received 25 August 1986/Accepted 5 December 1986

Type 1 fimbriated (mannose-specific) *Escherichia coli* cells bind to mannose residues on human polymorphonuclear leukocytes (PMN); this leads to phagocytosis of the bacteria. To identify the mannose-containing receptors on the PMN, the cells were surface labeled with ^{125}I and lysed in 0.5% Nonidet P-40, and the lysate was fractionated by affinity chromatography on a column of Sepharose-bound fimbriae. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography of the material eluted from the column with 500 mM methyl-α-mannoside revealed two radioactive bands of M_r 70,000 to 80,000 (gp70–80) and 100,000 (gp100). Another weak band of M_r 150,000 (gp150) was observed after prolonged exposure of the gel. Upon blotting of the glycoproteins separated by polyacrylamide gel electrophoresis and overlaying of the blots with concanavalin A, gp150 appeared as the major band. Membrane preparations of the PMN were enriched in gp70–80, gp100, and gp150, in comparison with the cell homogenates, further suggesting that these glycoproteins are surface components. Fractionation of the membrane preparations on the immobilized fimbriae followed by concanavalin A overlay of blots of the methyl-α-mannoside-eluted material revealed that gp150 was the major component in this fraction. The eluted fraction, obtained from a cell lysate (4.4 μg/ml), inhibited by 70% the agglutination of yeasts by the intact bacteria. Our results suggest that the three surface glycoproteins isolated by us serve as receptors for mannose-specific *E. coli* on PMN and may be involved in the lectin-mediated phagocytosis of the bacteria.

Opsonophagocytosis of bacteria is an extensively investigated process in which recognition between phagocytes and their target is mediated by antibody or complement or both (19, 40). Evidence obtained during the last few years has indicated that in the absence of opsonins, phagocytes may recognize bacteria by lectin-carbohydrate interactions (30, 37). The best-characterized system in which this recognition operates is between bacteria carrying mannose-specific lectins in the form of type 1 fimbriae that bind to mannose residues on the surface of mouse peritoneal macrophages or human polymorphonuclear leukocytes (PMN) with resultant stimulation of the phagocytes and internalization and killing of the bacteria (4, 5, 31, 38, 39). This process, which occurs in the absence of serum components, is known as nonopsonic phagocytosis (19).

Nonopsonic phagocytosis mediated by bacterial lectins may be of clinical relevance in tissues in which the opsonic activity is poor, such as the renal medulla (39), or when bacteria are unable to activate the alternative complement pathway (37). Little is known about the process of nonopsonic phagocytosis, and even less is known about the molecules that are on the surface of the phagocytes and are involved in the recognition of the bacteria. There are, however, strong indications that bacteria expressing mannose-specific lectins bind to mannose-containing glycoproteins on phagocytic cells. This is based on several lines of evidence. (i) Type 1 fimbrial lectins bind specifically to mannose, α-glycosides of mannose, and mannose-containing oligosaccharides but not to saccharides devoid of mannose (13). (ii) The same specificity pattern of inhibition is observed irrespective of the target cell used (e.g., yeasts [13], erythrocytes [13, 26], or BHK cells [12]). (iii) Whenever sugars other than mannose were examined, they inhibited poorly if at all the interaction of type 1 *Escherichia coli* with mouse peritoneal macrophages or PMN (4, 39). (iv) A very good correlation was found between the mannan-binding activity of the bacteria and the extent of their attachment to mouse peritoneal macrophages (5). (v) The finding that pretreatment of type 1 fimbriated bacteria with yeast mannan inhibited their attachment to mouse and human phagocytes, whereas pretreatment of the phagocytes did not have such an effect (4), shows that the receptor for the bacterial lectin is on the surface of the phagocytes. (vi) Since the only mannose-containing compounds in animal membranes are glycoproteins (32), the receptor for mannose-specific bacteria must belong to this class. In view of the above, we chose to use affinity chromatography on a column of immobilized type 1 fimbriae for the isolation of the receptors.

In this paper, we report the identification of surface glycoproteins of human PMN that mediate the attachment of *E. coli* bearing type 1 fimbriae.

MATERIALS AND METHODS

Materials. The Bolton and Hunter reagent was purchased from Amersham Radiochemical Center. Phenylmethylsulfonyl fluoride, polyvinylpyrrolidone (average molecular weight, 40,000), Nonidet P-40, Tween 20, pepstatin, leupeptin, peroxidase type II from horseradish, lactoperoxidase, 3,3′-diaminobenzidine, dextran (average molecular weight, 488,000), bovine serum albumin, and hemoglobin were obtained from Sigma Chemical Co. Nitrocellulose paper (BA-8S, 0.45μ) was from Schleicher & Schuell, Inc. Cyanogen bromide-activated Sepharose 4B and Percoll were obtained from Pharmacia Fine Chemicals. Methyl-α-mannoside (MeαMan) was from Pfanstiehl Laboratories, and concanavalin A was from Miles-Yeda.

Bacteria. *E. coli* serotype O83:K1:H4 strain 827, isolated from a patient with urinary tract infection, was grown

* Corresponding author.

without agitation in brain heart infusion broth (Difco Laboratories) for 48 h at 37°C, conditions which are optimal for the growth of bacteria expressing mannose-specific activity, as determined by a yeast aggregation assay (27). Only bacteria showing an aggregation rate higher than 10 U/min, at a concentration of 10^9 cells per ml, were used.

Isolation of type 1 fimbriae. Type 1 fimbriae were isolated by mechanical agitation of the bacterial suspension followed by repeated precipitation with 0.1 M $MgCl_2$ as previously described (11). The fimbriae were free of contaminants when examined by electron microscopy as well as by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate of a preparation that had been dissociated in saturated guanidine hydrochloride (11).

PMN. PMN were isolated from fresh human blood obtained from healthy donors. Briefly, the buffy coat (25 ml) was mixed with an equal volume of 5% dextran in phosphate-buffered saline (10 mM phosphate, 150 mM NaCl [pH 7.4]; PBS) and sedimented at unit gravity for 30 min at 37°C. The leukocyte-rich supernatant was collected, washed twice in PBS (without Ca^{2+} or Mg^{2+}), and resuspended in 50 ml of the same buffer. The leukocyte-rich fraction was further purified on a discontinuous Percoll gradient (18). A yield of 10^9 cells was obtained with a purity of 98% as determined by Giemsa staining (14).

Labeling. Bacteria were labeled with ^{125}I by using the Bolton and Hunter reagent (8) as follows. The reagent (1 mCi in 1 ml) was transferred into a siliconized tube, and the solvent was removed at room temperature by a gentle stream of nitrogen. The bacterial suspension (1 ml containing 10^9 cells) was added to the tube, which was then kept at 0°C for 15 min; subsequently, 1 ml of 200 mM glycine was added. The bacteria were washed with PBS and dialyzed for 24 h against PBS with several changes of buffer to remove the free iodine. Under these conditions, the specific activity of trichloroacetic acid-precipitable material was 11×10^{-3} cpm per bacterium. The labeled bacteria agglutinated yeast cells at the same rate as the unlabeled ones did.

PMN (2×10^8 cells per ml) were surface iodinated by the lactoperoxidase method (25). Labeling was at 4°C to reduce the recycling of cell surface components. The cells were washed several times with PBS to remove the free iodine. The specific activity obtained in the total cell lysate (see below) was approximately 1.5×10^3 cpm/μg of protein.

The fimbriae (1 mg/ml) were iodinated for 1 min at room temperature by the chloramine T method (20); free iodine was removed by extensive dialysis against PBS. The specific activity obtained was $(2.5 \pm 0.6) \times 10^6$ cpm/μg of protein. Fimbriae (20 μg/ml) with a yeast-aggregating activity higher than 5 U/min were used immediately after iodination.

Concanavalin A (0.5 mg/ml) was labeled with $Na^{125}I$ by using chloramine T as described above for the fimbriae; the specific activity obtained under these conditions was 10^6 cpm/μg of protein.

Cell lysates. Cell lysates of PMN were prepared as described by De Maio et al. (10). Briefly, 2×10^8 cells per ml were lysed for 30 min at 0°C in a solution containing 50 mM Tris hydrochloride (pH 8.1), 110 mM NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 0.5% Nonidet P-40, and the following protease inhibitors: leupeptin (20 μg/ml), pepstatin (5 μg/ml), and phenylmethylsulfonyl fluoride (10 μg/ml). The cell lysates were centrifuged at 100,000 × *g* for 1 h (Beckman 60 Ti rotor), and the supernatant was used for affinity chromatography. The yield of protein was 1.5 mg from 2×10^8 cells.

Cell homogenates. PMN (10^7 cells per ml) suspended in 10 mM Tris hydrochloride (pH 7.2)–150 mM NaCl–5 mM EDTA were disrupted by nitrogen cavitation (450 lb/in^2 for 30 min) (21). Samples were analyzed by gel electrophoresis, and the homogenate was used for plasma membrane isolation.

Plasma membrane isolation. For the isolation of plasma membranes, PMN (10^7 cells per ml) were disrupted as described by Klempner et al. (21) and the membrane fraction was isolated (10). Briefly, the cell homogenate (25 ml) was layered on a 42% (wt/vol) sucrose cushion (15 ml) and centrifuged at 100,000 × *g* for 1 h (Beckman SW 27 rotor). The fraction sedimenting at the sucrose-buffer interface was collected, diluted in the same buffer, and centrifuged at 100,000 × *g* for 1 h (Beckman 60 Ti rotor). By using the enzymatic membrane marker 5′-nucleotidase, assayed by the method of Tsai et al. (42), the plasma membrane fraction was at least 10-fold purified with a yield of about 70% (approximately 0.6 mg of protein from 5×10^8 cells). For affinity chromatography, the membrane pellet was suspended in 1 ml of 50 mM Tris hydrochloride (pH 8)–150 mM NaCl, solubilized with 1% Nonidet P-40, and centrifuged at 100,000 × *g* for 1 h (Beckman 65 rotor).

Binding assay. A PMN suspension (0.05 ml, 10^7 cells per ml) was added to each well of a disposable flexible microtiter plate (Dynatech Laboratories, Inc.). The cells were allowed to adhere at 37°C for 30 min in an atmosphere containing 5% CO_2, and the nonadherent cells were washed off. The cell monolayer, corresponding to approximately 10^5 PMN as calculated from experiments with radiolabeled cells, was incubated for 15 min in PBS containing 1% bovine serum albumin. The PBS-bovine serum albumin was removed, and the PMN were incubated with 0.05 ml of radiolabeled bacteria (10^8/ml) at 0°C for different time intervals (5, 15, and 30 min). In other experiments, the PMN were incubated with different concentrations of fimbriae at 0°C for 30 min. The unbound bacteria or fimbriae were removed, the plates were washed three times with PBS, and the individual wells were cut and counted. Parallel experiments were carried out in the presence of 250 mM MeαMan.

Affinity chromatography. Purified fimbriae (10 mg/g of dry beads) were coupled to cyanogen bromide-activated Sepharose 4B by the procedure given by the manufacturer. Under these conditions, 80% of the fimbriae were bound to the Sepharose, based on measurements of optical density at 280 nm of the solution of the fimbriae before and after coupling. The Nonidet P-40-soluble cell lysate or membrane fraction from human PMN was loaded on a small fimbria-Sepharose 4B column (1 ml, in a Pasteur pipette), equilibrated with column buffer (10 mM Tris hydrochloride [pH 7.2], 150 mM NaCl, 5 mM $CaCl_2$, 5 mM $MnCl_2$, 0.5% Nonidet P-40, 0.02% NaN_3). The column was washed until radioactivity of the effluent returned to the base-line value. The bound material was then eluted with 500 mM MeαMan in column buffer.

Sodium dodecyl sulfate-polyacrylamide gels electrophoresis. Samples were boiled for 2 min in electrophoresis sample buffer containing 5% (vol/vol) β-mercaptoethanol and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the Laemmli buffer system (23). As markers, the following proteins were used: myosin (M_r, 205,000), β-galactosidase (M_r, 116,000), phosphorylase *b* (M_r, 97,000), bovine serum albumin (M_r, 66,000), egg albumin (M_r, 45,000), and carbonic anhydrase (M_r, 29,000).

Protein blotting and concanavalin A overlay. After electrophoresis, the gels were immediately blotted (2 h) onto nitrocellulose filters (15, 16). The filters were quenched in 2% (wt/vol) polyvinylpyrrolidone–PBS (6). Horseradish peroxidase-concanavalin A overlay was carried out as de-

scribed by Hawkes (17). The blots were incubated with concanavalin A (50 μg/ml in 2% polyvinylpyrrolidone–PBS) for 1 h at room temperature, rinsed with PBS, and incubated with horseradish peroxidase (50 μg/ml in the same quencher buffer) for 1 h. The filters were then washed with 0.1% Tween 20–PBS for 1 h, with changes of buffer every 15 min. The bound peroxidase was detected by using 3,3'-diaminobenzidine (3%) and hydrogen peroxide (0.0012%). Concanavalin A-binding glycoproteins appear as brown bands 10 to 20 min after the addition of diaminobenzidine (room temperature). In experiments with ^{125}I-labeled concanavalin A, blots were quenched in 1% hemoglobin–PBS (10) for 2 to 16 h and incubated in 10 ml of 1% hemoglobin–PBS containing ^{125}I-labeled concanavalin A [(0.5 to 2.0) × 10^6 cpm/ml] for 2 h at room temperature. The filters were then washed with 0.1% Tween 20–PBS for 3 h, with changes of buffer every 30 min, and autoradiographed for 3 to 4 h with Curix RP2 Agfa X-ray films.

Protein assay. Protein was determined by the method of Lowry et al. (24) with bovine serum albumin as the standard.

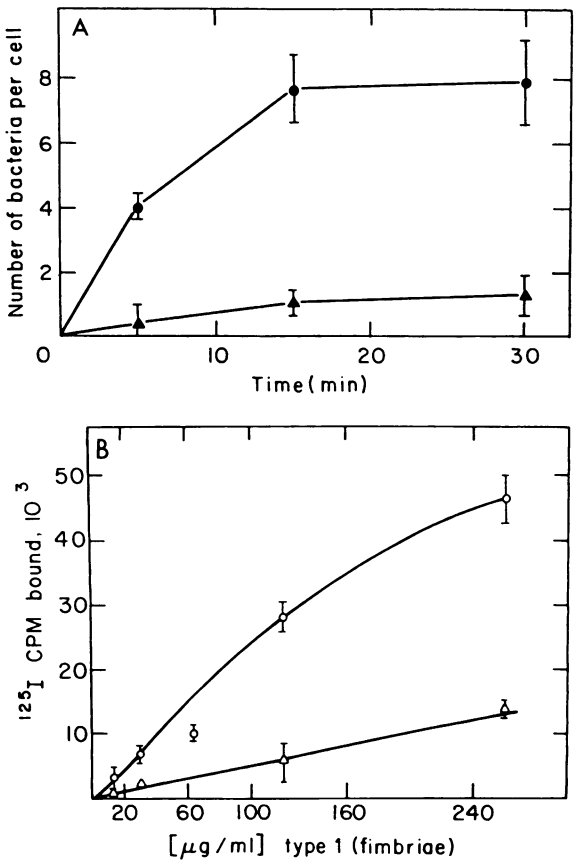

FIG. 1. Binding of ^{125}I-labeled *E. coli* 827 (A) and type 1 fimbriae (B) to human PMN. (A) Phagocytic cells (ca. 10^5 cells per microtiter well) were treated with ^{125}I-labeled bacteria (50 μl, 10^8 bacteria per ml) and then incubated for different intervals of time at 0°C. Symbols: ●, bacteria in buffer; ▲, bacteria in buffer containing 250 mM MeαMan. (B) Phagocytic cells were treated with ^{125}I-labeled fimbriae and then incubated for 30 min at 0°C. Symbols: ○, fimbriae in PBS; △, fimbriae in the presence of 250 mM MeαMan. At the end of incubation, unbound bacteria or fimbriae were removed by being washed with PBS, and the radioactivity was counted as described in the text. Each point is the mean of three experiments, and the bars represent standard deviations.

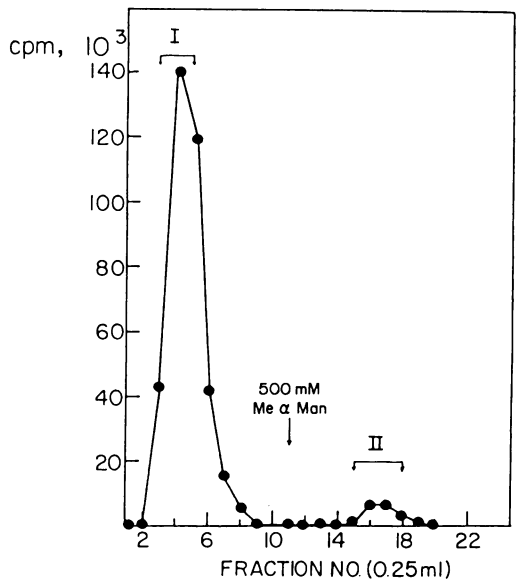

FIG. 2. Affinity chromatography on immobilized fimbriae of total cell lysates of ^{125}I-surface-labeled PMN: I, unbound fraction; II, fraction eluted with 500 mM MeαMan.

RESULTS

Binding. Binding of radiolabeled *E. coli* 827 reached a maximum within 10 min and at eight bacteria per cell (Fig. 1A). The binding of radiolabeled fimbriae to PMN was dose dependent and saturable (Fig. 1B). Both processes were mannose specific. These results are in line with previous studies (5, 39), suggesting that mannose-containing glycoproteins on PMN act as receptors for the fimbrial lectin.

Isolation of mannose-containing glycoproteins by affinity chromatography on immobilized fimbriae. The Nonidet P-40 lysate of PMN surface labeled with ^{125}I was loaded on a fimbriae-Sepharose 4B column. Two fractions were obtained, one containing the unbound material (fraction I) and the other containing the material eluted with 500 mM MeαMan (fraction II) (Fig. 2). The latter fraction made up 1 to 5% of the total TCA-precipitable radioactivity loaded on the column (Table 1). The total recovery of radioactivity from the column was between 59 and 74% of the material applied. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography revealed that the unfractionated material contained multiple bands, only a few of which were specifically bound to the fimbria column (Fig. 3). These were a band of M_r between 70,000 and 80,000 (gp70–80) and a minor band of M_r 100,000 (gp100). Upon longer exposure of the gel, a weak band of M_r 150,000 (gp150) appeared (Fig. 3, lane IIa).

To further identify the glycoproteins specifically bound to the fimbria column, we used the concanavalin A overlay technique. For this purpose, fraction II was electrophoresed, blotted onto nitrocellulose filters, and overlaid with horseradish peroxidase-concanavalin A. For comparison, the unfractionated lysate was similarly examined, except that the overlay was with ^{125}I-labeled concanavalin A. gp150 was the major band obtained upon ^{125}I-labeled concanavalin A overlay of blots of the unfractionated cell lysate (Fig. 4). With this preparation, it was possible to detect up to 15 bands after 3 to 4 h of exposure of the blot; more bands could be visualized after longer exposure (data not shown). Bind-

TABLE 1. Isolation of bacterium-binding glycoproteins from ^{125}I-labeled PMN by affinity chromatography on immobilized type 1 fimbriae

Expt	Amt applied[a] (10^5 cpm)	Amt unbound (10^5 cpm)	Amt (10^5 cpm) eluted with MeαMan (%)
1	10.0	7.3	0.10 (1.0)
2	6.4	3.4	0.36 (5.6)
3	15.0	10.0	0.70 (4.6)

[a] Counts precipitable by 10% trichloroacetic acid.

ing of concanavalin A to these glycoproteins was specific as demonstrated by its complete inhibition with 500 mM MeαMan (Fig. 4). gp150 was also the major concanavalin A-binding glycoprotein in fraction II isolated from the lysate (Fig. 4), although it appeared as a faint band on autoradiograms of ^{125}I-labeled fraction II (Fig. 3). gp70–80 and gp100 were also stained with horseradish peroxidase-concanavalin A but to a lesser extent than gp150 was; in addition, a band of M_r 45,000 was observed (Fig. 4).

To obtain further evidence of the cellular location of the concanavalin A-reactive glycoproteins, we analyzed membrane preparations from PMN by blotting as above. Compared with the total cell homogenate, gp150, gp100, and gp70–80 appeared as stronger bands on gels of the plasma membrane fraction, suggesting an enrichment of these glycoproteins (Fig. 5, lanes a and b). In another experiment, the Nonidet P-40-soluble fraction of the plasma membrane was loaded onto the fimbria column; fraction II obtained by elution of the column with MeαMan was analyzed by concanavalin A overlay. As with the total cell lysate, gp150 was the major concanavalin A-reactive band detected in this fraction (Fig. 5, lane c).

Inhibitory activity of isolated glycoproteins. Fraction II obtained by affinity chromatography of four different cell lysate preparations was pooled and dialyzed extensively against PBS to remove the MeαMan. This material, at a concentration of 4.4 μg/ml, inhibited 70% of the mannose-specific lectin activity of the bacteria as assayed by yeast aggregation (Fig. 6). No inhibition of yeast aggregation was obtained with the whole-cell lysate at a concentration of up to 80 μg of protein per ml.

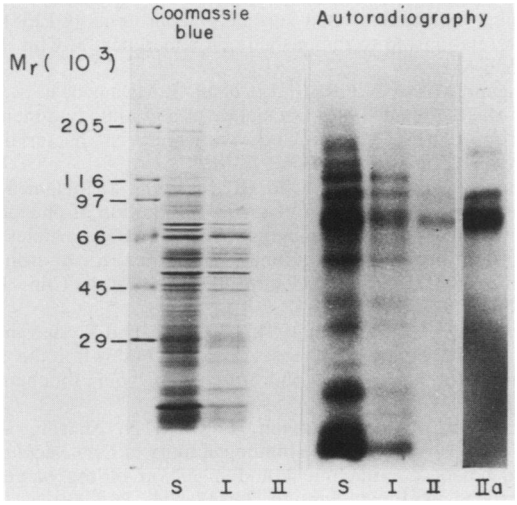

FIG. 3. Coomassie blue staining and autoradiography of ^{125}I-labeled cell lysate fractionated on the fimbria column. The peaks of radioactivity were precipitated with acetone and run in a 5 to 15% acrylamide gradient slab gel. Lanes: S, starting material (15,000 cpm); I, fraction unbound (10,000 cpm); II, fraction eluted with 500 mM MeαMan (2,000 cpm) after 24 h of exposure of gel; IIa, fraction II after 2 weeks of exposure. Markers: myosin (M_r, 205,000), β-galactosidase (M_r, 116,000), phosphorylase *b* (M_r, 97,000), bovine serum albumin (M_r, 66,000), egg albumin (M_r, 45,000), and carbonic anhydrase (M_r, 29,000).

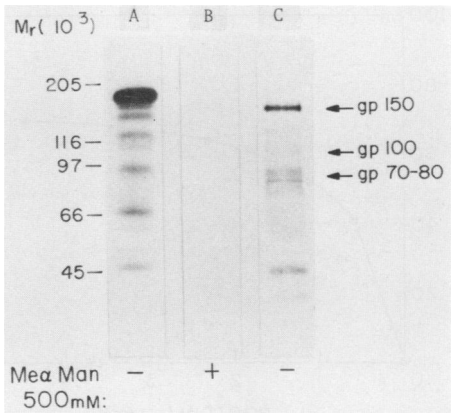

FIG. 4. Concanavalin A overlay of blot containing electrophoretically separated PMN lysate and fraction II derived from it. Proteins were electrophoresed on a 7.5% acrylamide slab gel, blotted onto nitrocellulose filters, and overlaid with lectin. The cell lysate (30 μg of protein) was overlaid with ^{125}I-labeled concanavalin A in the absence (lane A) and the presence (lane B) of 500 mM MeαMan. Fraction II (25 μg of protein) was overlaid with horseradish peroxidase-concanavalin A (lane C). The molecular weight markers were the same as those given in Fig. 3.

DISCUSSION

The aim of this study was to isolate the surface glycoproteins from PMN that are involved in the binding of mannose-specific bacteria to the cells described above in the absence of serum components. For this purpose, we used affinity chromatography on immobilized type 1 fimbriae. Only material bound and eluted with MeαMan is relevant since more than 85% of the attachment of the bacteria to PMN was inhibited by MeαMan (Fig. 1). The material bound and

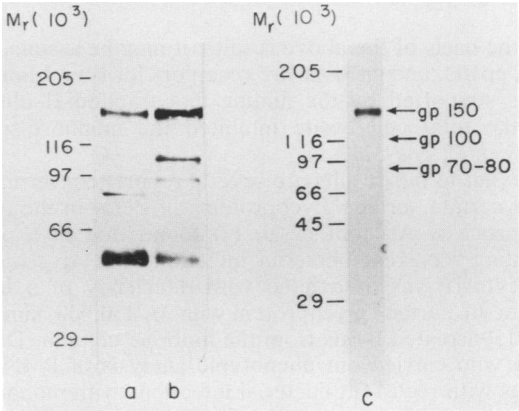

FIG. 5. Blot analysis with horseradish peroxidase-concanavalin A of PMN homogenate and membrane preparation. Proteins were electrophoresed (7.5% acrylamide slab gel), blotted onto nitrocellulose filters, and overlaid with lectin. Lanes: a, proteins (20 μg) of cell homogenates; b, proteins (20 μg) of membrane preparation; c, fraction II proteins (9 μg) eluted from the fimbria column of Nonidet P-40-soluble PMN membranes.

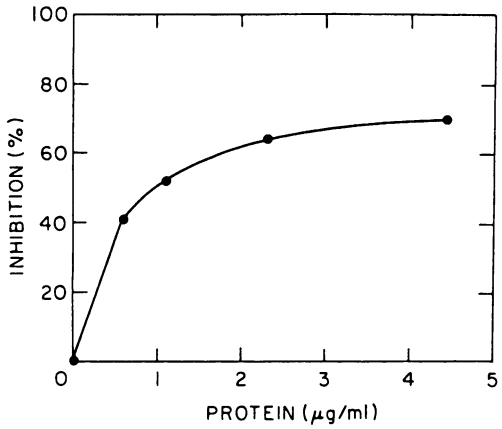

FIG. 6. Inhibition of *E. coli* 827 agglutination of yeasts by fraction II of total cell lysates eluted from the fimbria column. The material eluted with MeαMan from four fimbria columns was pooled and dialyzed extensively against PBS, and different volumes of this material were added to the yeast cell suspension before *E. coli* was added. Agglutinatination was monitored by aggregometry (13).

eluted with MeαMan (fraction II) was composed of the three surface glycoproteins gp150, gp100, and gp70–80, as demonstrated by experiments with cells labeled by the [^{125}I]lactoperoxidase method and by their enrichment in the plasma membrane fraction. gp150 was the strongest band observed after concanavalin A overlay analysis of the total cell lysate, of the isolated membranes, and of fraction II, suggesting that this glycoprotein is a major constituent on the surface of PMN that bind type 1 fimbriae (Fig. 3 and 4). The strong signal obtained with the concanavalin A overlay indicates that this glycoprotein may be highly glycosylated or may be present in many copies on the surface of PMN. This glycoprotein was, however, poorly labeled by the [^{125}I]lactoperoxidase method (Fig. 3). The major ^{125}I-labeled band of fraction II was gp70–80, which bound concanavalin A poorly. Concanavalin A overlay of the membrane preparation from PMN revealed a considerable enrichment of gp100 (Fig. 4, lane B); however, after fractionation on the fimbria column, this glycoprotein was difficult to detect in fraction II (Fig. 4, lane C). This may be due to differences in specificity between concanavalin A and the fimbrial lectin (13).

On the basis of the above results, it may be assumed that gp150, gp100, and gp70–80 are receptors for type 1 fimbriae. This is supported by the finding that fraction II obtained from the total cell lysate inhibited the mannose-specific activity of *E. coli* 827.

Relevant to our results are several reports concerning the role of certain surface glycoproteins of PMN in the phagocytic process. Arnaout et al. (2) found that in a patient presenting recurrent bacterial infection, defective opsonophagocytosis was associated with deficiency of a human granulocyte surface glycoprotein with M_r 150,000, similar to the gp150 isolated by us from the fimbriae column. Dana et al. (9), who carried out phenotypic analysis of PMN from patients with recurrent bacterial infections with monoclonal antibodies to these cells, found a deficiency of a human leukocyte membrane glycoprotein Mol (which has a subunit of 155 kilodaltons). Studies with monoclonal antibodies to Mol and to a similar surface antigen (Mac-1) on human and mouse leukocytes have shown that these antigens are closely associated with, or identical to, the receptor for the C3bi fragment of complement, designated CR3 (3, 7). Mac-1 appears to be the major concanavalin A-binding glycoprotein on the surface of human PMN (36). Preliminary experiments carried out by us showed that the anti-CR3 (Mac-1) monoclonal antibody inhibited the binding of *E. coli* 827 to human PMN by 25%.

Recent reports have described a genetic disorder of adhesion-dependent leukocyte function (clinically characterized by recurrent bacterial infection) which has been associated with deficiency on leukocytes of surface glycoproteins, such as CR3, lymphocyte function-associated antigen 1 (LFA-1), and the p150,95 molecule (1, 22, 41). CR3 is an important phagocyte receptor that triggers both phagocytosis and respiratory burst (33), but it is not known whether LFA-1 and p150,95 have similar functions. Ross et al. (34) showed that anti-CR3 and anti-LFA-1 inhibited normal phagocytosis by neutrophils of nonopsonized *Staphylococcus epidermidis* and suggested that the absence of this nonimmune recognition of bacteria by the neutrophils of the patients may be one of the reasons for their increased susceptibility to bacterial infections.

These reports, together with our findings, suggest that gp150, the major concanavalin A-binding glycoprotein isolated from the fimbria column, may be identical to CR3. Thus, CR3, known to be involved in phagocytosis of opsonized particles (33), may serve in addition as one of the receptors for the lectin and mediate phagocytosis of mannose-specific bacteria in the absence of opsonins.

ACKNOWLEDGMENTS

We thank Halina Lis for her critical reading of the manuscript and her helpful suggestions and Dvorah Ochert for editorial assistance.

LITERATURE CITED

1. **Anderson, D. C., F. C. Schmalstieg, M. A. Arnaout, S. Kohl, M. F. Tosi, N. Dana, G. J. Buffone, B. J. Hughes, B. R. Brinkley, W. D. Dickey, J. S. Abramson, T. Springer, L. A. Boxer, J. M. Hollers, and C. W. Smith.** 1984. Abnormalities of polymorphonuclear leukocyte function associated with heritable deficiency of high molecular weight surface glycoproteins (GP138): common relationship to diminished cell adherence. J. Clin. Invest. **74**:536–551.
2. **Arnaout, M. A., J. Pitt, H. J. Cohen, J. Melamed, F. S. Rosen, and H. R. Colten.** 1982. Deficiency of a granulocyte-membrane glycoprotein (GP150) in a boy with recurrent bacterial infections. N. Engl. J. Med. **306**:693–699.
3. **Arnaout, M. A., R. F. Todd III, N. Dana, J. Melamed, S. F. Schlossman, and H. R. Colten.** 1983. Inhibition of phagocytosis of complement C3 or immunoglobulin G-coated particles and of C3bi binding by monoclonal antibodies to a monocyte-granulocyte membrane glycoprotein (M01). J. Clin. Invest. **72**:171–179.
4. **Bar-Shavit, Z., R. Goldman, D. Mirelman, and N. Sharon.** 1977. Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi*. Biochem. Biophys. Res. Commun. **78**:455–460.
5. **Bar-Shavit, Z., R. Goldman, I. Ofek, N. Sharon, and D. Mirelamn.** 1980. Mannose-binding activity of *Escherichia coli*: a determinant of attachment and ingestion of the bacteria by macrophages. Infect. Immun. **29**:417–424.
6. **Bartles, J. R., and A. L. Hubbard.** 1984. ^{125}I-Wheat germ agglutinin blotting: increased sensitivity with polyvinylpyrrolidone quenching and periodate oxidation/reductive phenylamination. Anal. Biochem. **140**:284–289.
7. **Beller, D., T. Springer, and R. Schreiber.** 1982. Anti Mac-1 selectively inhibits the mouse and human type three complement receptor. J. Exp. Med. **156**:1000–1009.
8. **Bolton, A., and W. Hunter.** 1973. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem. J. **133**:529–539.

9. **Dana, N., R. F. Todd III, J. Pitt, T. A. Springer, and M. A. Arnaout.** 1984. Deficiency of a surface membrane glycoprotein (M01) in man. J. Clin. Invest. **73**:153–159.
10. **De Maio, A., H. Lis, J. M. Gershoni, and N. Sharon.** 1986. Identification of glycoproteins that are receptors for peanut agglutinin on immature (cortical) mouse thymocytes. FEBS Lett. **194**:28–32.
11. **Eshdat, Y., F. J. Silverblatt, and N. Sharon.** 1981. Dissociation and reassembly of *Escherichia coli* type 1 pili. J. Bacteriol. **148**:308–314.
12. **Firon, N., D. Duksin, and N. Sharon.** 1985. Mannose-specific adherence of *Escherichia coli* to BHK cells that differ in their glycosylation patterns. FEMS Microbiol. Lett. **27**:161–165.
13. **Firon, N., I. Ofek, and N. Sharon.** 1983. Carbohydrate specificity of the surface lectins of *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. Carbohydr. Res. **120**:235–249.
14. **Garvey, J. S., N. E. Cremer, and D. H. Sussdorf.** 1977. Methods in immunology, 3rd ed., p. 526. Benjamin, Reading, Mass.
15. **Gershoni, J. M., F. E. Davis, and G. E. Palade.** 1985. Protein blotting in uniform or gradient electric fields. Anal. Biochem. **144**:32–40.
16. **Gershoni, J. M., and G. E. Palade.** 1983. Protein blotting: principles and applications. Anal. Biochem. **131**:1–15.
17. **Hawkes, R.** 1982. Identification of concanavalin A-binding proteins after sodium dodecyl sulfate-gel electrophoresis and protein blotting. Anal. Biochem. **123**:143–146.
18. **Hjorth, R., A. K. Jonsson, and P. Vretblad.** 1981. A rapid method for purification of human granulocytes using Percoll: a comparison with dextran sedimentation. J. Immunol. Methods **43**:95–101.
19. **Horwitz, M. A.** 1982. Phagocytosis of microorganisms. Rev. Infect. Dis. **4**:104–123.
20. **Hunter, W. M., and I. C. Greenwood.** 1962. Preparation of iodine-131-labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
21. **Klempner, M. S., R. B. Mikkelson, D. H. Corfman, and J. Andre-Schwartz.** 1980. Neutrophil plasma membranes. I. High-yield purification of human neutrophil plasma membrane vesicles by nitrogen cavitation and differential centrifugation. J. Cell Biol. **86**:21–28.
22. **Kohl, S., T. Springer, F. C. Schmalstieg, L. S. Loo, and D. C. Anderson.** 1984. Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM1 deficiency. J. Immunol. **133**:2972–2978.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
25. **Marchalonis, J. L., R. E. Cone, and V. Santer.** 1971. Enzymic iodination: a probe for accessible surface proteins of normal and neoplastic lymphocytes. Biochem. J. **124**:921–927.
26. **Neeser, J.-R., B. Koellreutter, and P. Wuersch.** 1983. Oligomannoside-type glycopeptides inhibiting adhesion of *Escherichia coli* strains mediated by type 1 pili: preparation of potent inhibitors from plant glycoproteins. Infect. Immun. **52**:428–436.
27. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22**:247–254.
28. **Ofek, I., and E. H. Beachey.** 1980. General concepts and principles of bacterial adherence in animals and man, p. 1–29. *In* E. H. Beachey (ed.), Bacterial adherence. Chapman & Hall, Ltd., London.
29. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623–625.
30. **Ofek, I., and A. Perry.** 1985. Molecular basis of bacterial adherence to tissues. p. 7–13. *In* S. E. Mergenhagen and B. Rosen (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
31. **Perry, A., I. Ofek, and F. J. Silverblatt.** 1983. Enhancement of mannose-mediated stimulation of human granulocytes by type 1 fimbriae aggregated with antibodies on *Escherichia coli* surfaces. Infect. Immun. **39**:1334–1345.
32. **Rauvala, H., and J. Finne.** 1979. Structural similarity of the terminal carbohydrate sequence of glycoproteins and glycolipids. FEBS Lett. **97**:1–8.
33. **Ross, G. D., J. A. Cain, and P. J. Lachmann.** 1985. Membrane complement receptor type three (CR_3) has lectin-like properties analogous to bovine conglutinin and functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. J. Immunol. **134**:3307–3315.
34. **Ross, G. D., R. A. Thompson, M. J. Walport, T. A. Springer, J. V. Watson, R. H. R. Ward, J. Lida, S. L. Newman, R. A. Harrison, and P. J. Lachmann.** 1985. Characterization of patients with an increased susceptibility to bacterial infections and a genetic deficiency of leukocyte membrane complement receptor type3 and the related membrane antigen LFA-1. Blood **66**:882–890.
35. **Rottini, G., F. Cian, M. R. Soranzo, R. Albrigo, and P. Patriarca.** 1979. Evidence for the involvement of human polymorphonuclear leucocyte mannose-like receptors in the phagocytosis of *Escherichia coli*. FEBS Lett. **105**:307–312.
36. **Schmalstieg, F. C., H. E. Rudloff, and D. C. Anderson.** 1986. Binding of the adhesive protein complex (LFA-1/Mac-1/p150,95) to concanavalin A. J. Leukocyte Biol. **39**:193–203.
37. **Sharon, N.** 1984. Surface carbohydrate and surface lectins are recognition determinants in phagocytosis. Immunol. Today **5**:143–147.
38. **Sharon, N., and I. Ofek.** 1986. Mannose specific bacterial surface lectins, p. 55–81. *In* D. Mirelman (ed.), Microbial lectins and agglutinins: properties and biological activity, John Wiley & Sons, Inc., New York.
39. **Silverblatt, F. J., J. S. Dreyer, and S. Schauer.** 1979. Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. Infect. Immun. **214**:218–223.
40. **Silverstein, S. C., R. M. Steinman, and Z. A. Cohn.** 1977. Endocytosis. Annu. Rev. Biochem. **46**:669–722.
41. **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalstieg, and D. C. Anderson.** 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. J. Exp. Med. **160**:1901–1918.
42. **Tsai, C.-M., K.-Y. Chen, and E. S. Canellakis.** 1975. Isolation and characterization of the plasma membrane of L-1210 cells: iodination as a marker for the plasma membrane. Biochim. Biophys. Acta **401**:196–212.