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Binding of Mucin by *E. coli* from Human Gut

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Cells of *E. coli* isolates from the gut of healthy volunteers (N=5) and patients with Crohn's disease (N=5) and laboratory *E. coli* strain DH5α bound mucin *in vitro* in similar amounts ranging from 0.02 to 0.12 mg/mg of bacterial dry weight. Binding was evaluated by the decrease in optical absorption of mucin solution at 214 nm after incubation with bacteria. Detailed analysis of mucin binding by one of isolates showed that during incubation of 0.09 mg/ml bacteria in 0.15 M NaCl containing 0.1 mg/ml mucin at 25°C, maximum binding was reached in 30 min, while in the presence of 14 mM α-methyl mannoside, mucin binding decreased by 46% (p<0.05). Confocal microscopy revealed intensive binding of FITC-labeled mucin to the surface of a small number of bacterial cells. Mucin binding did not significantly affect zeta potential of bacteria and their energetic status assessed by ATP content; at the same time, ATP content in the extracellular environment slightly increased.

Key Words: *mucin binding; E. coli from human gut*

Adhesion of bacterial cells mediated by mucins secreted by goblet cells of the gut is an important stage of bacterial colonization of the intestinal mucosa [9]. Mucins are high-molecular-weight glycoproteins consisting of 80% carbohydrates (galactose, N-acetylglucosamine, N-acetylgalactosamine, fucose, and sialic acids) and 20% protein [5,9]. They are supposed to act as a decoy molecules preventing the interaction of bacterial adhesins with receptors on the surface of epithelial cells [9,11].

It can be assumed that affinity for mucin plays a role not only in adhesion, but also in binding of free molecules of glycoprotein to bacteria, but this interaction for *E. coli* from the human gut has not been studied. *E. coli* is usually isolated from feces, but the increasing attention is not attracted to the parietal microflora isolated from lavage fluid and biopsy specimens obtained endoscopically, *e.g.* in Crohn's disease (CD) [1].

We studied mucin adsorption by *E. coli* bacteria isolated from the human gut.

MATERIALS AND METHODS

The laboratory *E. coli* strain DH5α and *E. coli* isolates obtained from patients with CD treated at the Central Research Institute of Gastroenterology (Moscow) and healthy people were used in the study. Bacteria were isolated from bioptates and lavage fluid from the ileum of patients and from feces and isolated colonies were identified using Bruker Microflex mass spectrometer and Biotyper software (Bruker). The bacteria were grown in a liquid LB medium at 37°C (200 rpm) overnight to receive suspensions. The bacterial cells were washed carefully with 0.15 M NaCl and normalized at optical density of 540 nm. The optical absorption unit of the bacterial suspension corresponded to 0.2 mg/ml of air-dry bacterium weight and contained 8×10^8 CFU/ml.

Mucin from porcine stomach (type III; Sigma-Aldrich) was previously purified by gel filtration on a Sephadex G-200 column, high-molecular-weight fractions demonstrating positive staining by the Schiff

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method [4] were isolated and lyophilized. Mucin concentration was determined spectrophotometrically by absorption at 214 nm (A^{214}) and by analytical gel filtration using calibration curves (0.01-0.10 mg/ml). Analytical chromatography of mucin samples (200 µl) was conducted on a BioFox 17 SEC column (10×300 mm) at a flow rate of 0.5 ml/min in a solution of 0.15 M NaCl.

Bacterial suspension (0.09 mg of dry weight/ml) was added to 1 ml solution of purified mucin (0.1 mg/ml) in 0.15 M NaCl and incubated for 1 h at 25°C (test samples); control samples contained no mucin. At the end of incubation, the cells were precipitated by centrifugation (20 min, 900*g*) and mucin concentration in the supernatant was measured using a solution of 0.1 mg/ml mucin in 0.15 M NaCl as the reference sample. In some experiments, different temperatures (4-37°C) and different incubation time (0.25-1.5 h) were used and 14 mM α-methylannoside (α-MeMan) was added.

Zeta potential of mucin and bacteria was determined with dynamic laser scattering (Malvern Zetasizer Nano ZS). For ATP assay, the luminometer LYUM-1 with Lumtek luciferin-luciferase kits was used. The intracellular content of ATP was evaluated after cell destruction with 10-fold excess of DMSO, the extracellular content was measured in the supernatant after cell precipitation from the suspension by centrifugation. Osmotic shock was caused by incubation of bacteria in 0.015 M NaCl for 15 min [10].

For confocal microscopy, mucin was preliminary conjugated with FITC (FITC-mucin) [3]. Samples of control suspensions of bacteria and samples of bacterial suspensions after incubation with FITC-mucin were placed in a sealed chamber. After sedimentation of bacteria, the preparations were analyzed in a Nikon Eclipse E800 confocal laser-scanning microscope. Fluorescence of FITC-mucin was activated by an argon laser (λ=488 nm).

Statistical processing of the results was conducted using Statistica 6.0. The results were expressed as the mean (n=3-5) and standard deviation. To compare the parameters, the Student's t test or the Mann—Whitney test was used, the differences were considered significant at $p<0.05$.

RESULTS

The absorption spectrum of the commercial mucin had two peaks (Fig. 1). Purification of mucin by gel filtration allowed separation of a low molecular fraction with maximum absorption at 260 nm (A_{260}), which did not stain specifically in accordance with the Schiff method. In the purified mucin, the absorption of A_{260} was insignificant (Fig. 1),

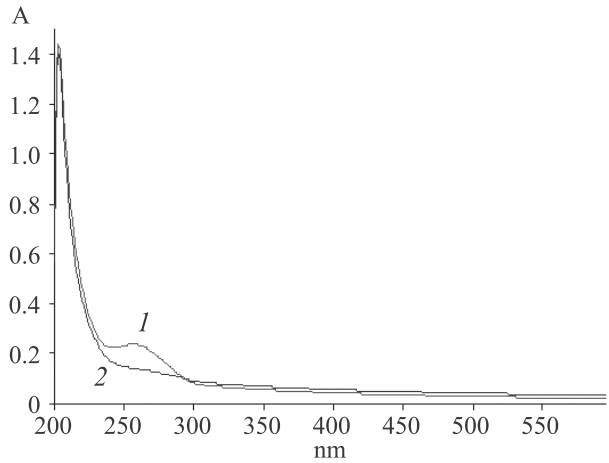

Fig. 1. Spectra of optical absorption of solutions of commercial (*1*) and purified (*2*) mucin in 0.15 M NaCl (concentration of mucin 0.1 mg/ml).

which allowed further determination of the glycoprotein concentration in A_{214}.

The amount of bacteria-bound mucin varied from 0.021 to 0.124 mg/mg of dry weight (Table 1). For the isolates from patients, the median was 0.035 mg/mg, and for isolates from healthy people 0.043 mg/mg, there were no significant differences between these groups ($p>0.05$, Mann—Whitney test). For further studies, the isolate of SharL1, which has the highest ability of mucin binding, was chosen.

In accordance with the analytical gel filtration, the bacteria of the SharL1 isolate bound mucin in an amount of 0.07±0.02 mg/mg, which corresponded to the obtained results (Table 1). Confocal microscopy

TABLE 1. Binding of Purified Mucin of *E. coli* from Healthy People, Patients with CD and Laboratory Strain (Lab) for the Estimation of the Loss of Absorption of Mucin (A_{214}) after Incubation with Bacteria

Isolate	Source	Mucin binding, mg/mg
Healthy people		
z8	Lavage	0.021±0.006
92k1	Feces	0.032±0.007
92k5	Feces	0.124±0.001
13k2	Feces	0.043±0.0091
40k1	Feces	0.094±0.006
CD		
k3	Lavage	0.023±0.015
B3	Lavage	0.021±0.001
AnB2	Bioptate	0.053±0.008
BruB2	Bioptate	0.035±0.003
SharL1	Lavage	0.086±0.015
DH5α	Lab	0.079±0.003

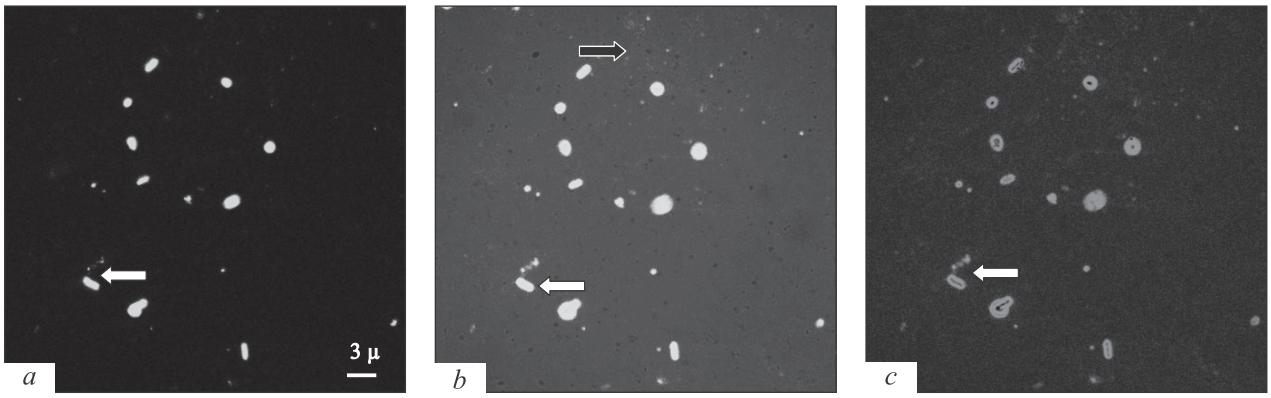

Fig. 2. Isolate SharL1 of *E. coli* after incubation with FITC-mucin. Confocal microscopy (*a*) and transmitted light (*b*, *c*). White arrows show bacteria that bound FITC-mucin, black arrows unbound bacteria.

showed that the cells of the SharL1 isolate significantly differ in the binding of FITC-mucin (Fig. 2, *a*, *b*). On the surface of most of the cells, FITC-mucin was not detected, but some bacteria bound mucin intensely. A kind of "cocoon" was formed around such cells, and the processing of images in the Adobe Photoshop CC program with the conversion of the color difference into contrast of brightness made it possible to identify the bacteria inside the "cocoon" (Fig. 2, *c*).

The increase of temperature from 25 to 37°C at incubation of bacteria for 1 h in the solution of mucin did not lead to a significant change of the amount of bound mucin. At 25°C, the adsorption reached a maximum after 30 min and did not change significantly with further incubation (up to 1.5 h). In the presence of 14 mM α-MeMan, the amount of adsorbed mucin decreased by 54±22% of the control values (without α-MeMan) ($p<0.05$, *t* test).

Zeta potential of the bacteria was -(44.2±2.0) mV, which agrees with published data (-49 mV at pH 4.99 [8]). After the adsorption of mucin that has zeta-potential -(36.2±1.0) mV, the surface charge of bacteria practically did not change and was -(41.2±5.1) mV.

TABLE 2. The Content of ATP (nM) in the Control and Mucin-Treated Bacteria before (0.15 M NaCl) and after (0.015 M NaCl) Osmotic Shock

Experimental condition	*E. coli*+NaCl (control)	*E. coli*+mucin (experiment)
0.15 M NaCl		
intracellular	412±42	405±7
extracellular	0.78±0.08	1.25±0.03*
0.015 M NaCl		
intracellular	267±18	246±4
extracellular	9.14±1.00	12.0±0.7*

Note. *$p<0.05$ in comparison with the control (*t* test).

Binding of mucin did not affect the intracellular content of ATP, but increased ATP release in the extracellular environment, including after osmotic shock (Table 2).

Different bacteria — *Bacteroides fragilis* [6], *Streptococcus pyogenes* [7], *Aeromonas sp.* [2] have the ability to adsorb mucin *in vitro*. At incubation in solution of mucin for 1-2 hours at 37°C, maximal binding of mucin by *B. fragilis* bacteria was achieved at a mucin concentration of 0.2 mg/ml, but only about 1.5% bacterial cells bound mucin [6]. In our experiments, at similar conditions (0.1 mg/ml of mucin, 25°C, 60 min), all investigated *E. coli* adsorbed mucin. There were no significant differences between isolates from healthy individuals and patients with CD. Confocal microscopy revealed heterogeneity of cells by the ability to bind mucin within one isolate, as in the case of *B. fragilis* that indicates the need for studies at the level of individual cells. The inhibitory effect of α-MeMan indicates the involvement of mannose-sensitive adhesins, which specifically bind to mannose-containing structures both in mucin and in the surface of epithelial cells [9]. Since these adhesins are localized on type 1 fimbria [12], the increase in the size of bacteria coated with FITC-mucin (Fig. 2) may be due to binding of mucin to the terminal portions of the pili. Adsorption of mucin did not change the zeta potential of bacterial cells and did not affect the energy status of *E. coli* by intracellular ATP. A small but significant increase in the extracellular content of ATP in a suspension of bacteria that adsorbed mucin, including after osmotic shock deserves additional studies. The effect can be associated with the reaction of bacteria to binding of mucin and therefore depends on the nature of the proteins that interact with the glycoprotein.

Thus, isolates of *E. coli* from the human gut are capable of binding mucin, including through mannose-sensitive interactions. There were no significant differ-

ences in binding of mucin between bacteria isolated from healthy people and patients with CD. Only a small fraction of the cells of one isolate binds FITC-mucin in a detectable amount. Adsorption of mucin does not affect the zeta potential of bacteria and their energy status, but enhances ATP release in the extracellular space.

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