Adherence of *Ureaplasma urealyticum* to Human Erythrocytes

ANN-BRITT SAADA,¹ YEHUDIT TERESPOLSKI,¹ AMIRAM ADONI,² AND ITZHAK KAHANE¹*  

Department of Membrane and Ultrastructure Research, Hebrew University-Hadassah Medical School, Jerusalem 91010,¹ and Department of Obstetrics and Gynecology, Hadassah University Hospital, Mt. Scopus, Jerusalem,² Israel

Received 16 July 1990/Accepted 9 October 1990

*Ureaplasma urealyticum* (four serotypes and two clinical isolates) were metabolically labeled with radioactive methionine to a high specific activity. Labeling allowed the study of the mechanism of adherence to human erythrocytes. The adherence mechanism was complex and partially mediated by proteinaceous surface components. The binding sites on the erythrocytes were partially sensitive to neuraminidase treatment, and adherence was inhibited by glycoporin and dextran sulfate, indicating recognition of sialyl residues and sulfated compounds.

The ability to adhere is a prerequisite for pathogenesis by many microorganisms (2). The adherence mechanisms of a number of mycoplasmas, particularly that of *Mycoplasma pneumoniae*, have been extensively investigated (1, 5-9, 11-13, 15). It has been shown that the adherence of *M. pneumoniae* to both respiratory cells and erythrocytes utilizes similar mechanisms. For example, *M. pneumoniae* proteinaceous components mediate adherence to neuraminidase-sensitive regions on both respiratory epithelium and erythrocytes (6). Thus, the model system using erythrocytes has been used to study the adherence mechanisms of *M. pneumoniae*, *Mycoplasma gallisepticum*, and other mycoplasmas (1, 6, 12, 13).

*Ureaplasma urealyticum* is an inhabitant and possible pathogen of the human urogenital tract (18). The organisms have been observed adhering to various cells, including epithelial cells from urethra (18), spermatozoa (3), and animal cells in culture (8). However, the adherence mechanism of *U. urealyticum* is yet to be elucidated. The lack of quantitative studies has been mostly due to the difficulty of labeling the organisms to high enough specific activity by using the procedures that were successful with other mycoplasmas, e.g., incorporation of radioactive fatty acids (1) or L-[35S]methionine (7, 14). We hereby present an adaptation of the method for metabolic labeling *Escherichia coli* with L-[35S]methionine to label *U. urealyticum* cells after growth. By using this method, we obtained cells sufficiently labeled to allow evaluation and characterization of some features of their adherence mechanism.

*U. urealyticum* serotypes 2, 3, 7, and 8 and two clinical isolates were grown in 1 liter of medium containing PPLO broth, 10% horse serum, 5% fresh yeast extract, and 6.6 mM urea (17). The organisms were harvested and washed twice in 0.25 M NaCl, as previously described (17). The pellet was suspended in 0.8 ml of Hanks buffered salt solution from which sulfate-containing salts were omitted and replaced with NaCl (HBSS-M). Subsequently, 4 μl of L-amino acid mixture (alanine, 0.4 mM; arginine, 0.6 mM; asparaginase, 0.32 mM; aspartate, 0.3 mM; glycine, 0.13 mM; isoleucine, 0.3 mM; leucine, 0.3 mM; lysine, 0.3 mM; phenylalanine, 0.3 mM; proline, 2.0 mM; serine, 4.0 mM; threonine, 0.3 mM; tryptophan, 0.1 mM, tyrosine, 0.1 mM; valine, 0.3 mM) (4) and L-[35S]methionine (0.5 mM; Amersham, Amersham, England) were added. The suspension was divided into four aliquots and incubated for 2 h at 37°C, and then 1 ml of HBSS-M containing 0.33 mM methionine (Sigma Chemicals) was added. After a 10-min chase, the suspension was centrifuged for 10 min at 15,000 rpm at 4°C in a microcentrifuge (Mikroliter; Hettich, Tuttingen, Federal Republic of Germany). The supernatant was discarded, and the pellet was washed twice in methionine-containing HBSS-M and suspended to a concentration of 50 μg of protein per ml in buffer A (50 mM Tris, 100 mM NaCl, 1 mM CaCl₂, pH 7.2) by forcing the pellet through a 26-gauge needle five times. The suspension was used in adherence experiments or solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 8% acrylamide gels (10).

The labeling conditions were selected after a series of experiments, including determination of optimal labeling time and washings. After 2 h of incubation, the cells were sufficiently labeled (about 16 × 10⁶ cpn/mg of protein) to enable assessment of adherence and to receive a sharp fluorograph from X-ray films exposed (3 to 7 days at -70°C) to labeled proteins in dried gels after SDS-PAGE.

Urea is an essential growth factor of *U. urealyticum*, and hydrolysis of urea by its urease is involved in ATP generation (16, 18). Therefore, the influence of urea on the labeling procedure was investigated. The addition of 50 mM urea to the labeling mixture did not alter the amount of incorporation or the electrophoretic pattern of the labeled proteins.

The different strains were labeled to the same extent, and the majority of the ureaplasmal polypeptides were labeled, as judged from the comparison of silver stain and fluorograph (Fig. 1). The advantages in using a metabolic label with ureaplasmas are as follows. (i) The elimination of labeling contaminating medium components is a serious problem with these fastidious organisms requiring a complex growth medium containing serum (14, 18). (ii) Protein labeling is preferred over fatty acid labeling, since it allows tracking of proteinaceous components involved in adherence.

The availability of metabolically labeled *U. urealyticum* with a high specific activity enabled us to assess some features of the ureaplasmal mechanism of adherence to human erythrocytes.

Adherence was assayed in plastic tubes as previously described (1). Briefly, the assay contained 0.05 ml of buffer A, 0.05 ml of 2% fresh human erythrocytes, and 0.1 ml of a
NOTES

serotypes different remaining The solution Amplify markers at tion ureaplasmas of cytotest tubes subtracted from the values obtained from (1).

performed reached mentions culture urealyticum tract. These which originated ureaplasmas. After 30 min adherence was decreased to 12% after 1 h with papain (0.5 μg/ml), chymotrypsin (0.2 μg/ml), or trypsin (2.5 to 5.0 μg/ml) adhered 33 to 39% less than cells treated with these proteases in the presence of their respective inhibitor (iodoacetic acid, 10 mM; phenylmethylsulfonyl fluoride, 0.5 mM; 1-chloro-3-tosylamide-7-amino-2-heptanone, 30 μg/ml). Proteolytic digestion, as judged by SDS-PAGE, resulted in the loss of some minor polypeptide bands of molecular mass above 95 KDa (data not shown).

Sialyl receptors on the surface of host cells have been reported to mediate adherence of many mycoplasmas (5, 6, 12). We investigated this in ureaplasmas, using neuraminidase-treated erythrocytes. The findings that asialo-erythrocytes (1) bound 29% ± 3% fewer ureaplasmas confirmed this to some extent. The ability of glycoporphin, but not asialoglycoporphin, to interfere with adherence further strengthened the aforementioned result (Table 1).

The sialoglycoproteins, α-1-acid glycoprotein and fetuin, interfered less than glycoporphin with the adherence of U. urealyticum (Table 1). Bovine serum albumin was included in these experiments as a negative control (Table 1). These results and the finding that free N-acetylneuraminic acid (2 mM) did not interfere with adherence indicate the importance of the linkage in which the sialic acid residues are bound. This is common to other microbial adhesins and was previously reported for M. pneumoniae and M. gallisepticum (5, 15).

Krivan et al. (9) recently reported the importance of sulfatide receptors in M. pneumoniae binding. The similarity with U. urealyticum was shown by our findings that the addition of 10 μg of dextran sulfate (Mw, ~500,000) in the

The ability to adhere was not influenced by the addition of 50 mM urea or by the urease inhibitor Flurofamide (50 μM) (Norwich Eaton Pharmaceuticals). This indicates that the ureaplasmal urease, which is essential in U. urealyticum energy metabolism (16, 18), does not participate in the adherence process.

The optimal temperature for attachment was 37°C. The decrease of adherence by 63% ± 3% at a lower temperature (0°C) may be indicative of an alteration of the metabolic state of the organism or of the mainly nonhydrophobic nature of these interactions. Heat treatment (30 min at 55°C) of the labeled ureaplasma prior to the adherence assay resulted in the loss of 18% ± 2% binding capacity. These findings indicate the involvement of ureaplastic proteins. The results were further confirmed by the finding that labeled ureaplasmas (50 μg of protein per ml) treated for 1 h with papain (0.5 μg/ml), chymotrypsin (0.2 μg/ml), or trypsin (2.5 to 5.0 μg/ml) adhered 33 to 39% less than cells treated with these proteases in the presence of their respective inhibitor (iodoacetic acid, 10 mM; phenylmethylsulfonyl fluoride, 0.5 mM; 1-chloro-3-tosylamide-7-amino-2-heptanone, 30 μg/ml). Proteolytic digestion, as judged by SDS-PAGE, resulted in the loss of some minor polypeptide bands of molecular mass above 95 KDa (data not shown).

Sialyl receptors on the surface of host cells have been reported to mediate adherence of many mycoplasmas (5, 6, 12). We investigated this in ureaplasmas, using neuraminidase-treated erythrocytes. The findings that asialo-erythrocytes (1) bound 29% ± 3% fewer ureaplasmas confirmed this to some extent. The ability of glycoporphin, but not asialoglycoporphin, to interfere with adherence further strengthened the aforementioned result (Table 1).

The sialoglycoproteins, α-1-acid glycoprotein and fetuin, interfered less than glycoporphin with the adherence of U. urealyticum (Table 1). Bovine serum albumin was included in these experiments as a negative control (Table 1). These results and the finding that free N-acetylneuraminic acid (2 mM) did not interfere with adherence indicate the importance of the linkage in which the sialic acid residues are bound. This is common to other microbial adhesins and was previously reported for M. pneumoniae and M. gallisepticum (5, 15).

Kriván et al. (9) recently reported the importance of sulfatide receptors in M. pneumoniae binding. The similarity with U. urealyticum was shown by our findings that the addition of 10 μg of dextran sulfate (Mw, ~500,000) in the

FIG. 1. Electrophoretic pattern of labeled polypeptides from different serotypes of U. urealyticum. Serotypes 2, 3, 7, and 8 were labeled with L-[35S]methionine for 2 h. After denaturation and reduction, 10 μg of labeled proteins was loaded in each slot and subjected to SDS-PAGE. One part of the gel was soaked with Amplify solution (Amersham), dried, and exposed to X-ray film (a). The remaining part was stained with silver (b). Molecular mass markers were run in the flanking slots.

FIG. 2. Adherence of different serotypes of U. urealyticum to human erythrocytes. Serotypes 2 ( ), 3 ( ), 7 ( ), and 8 ( ) labeled with radioactive methionine were assayed for adherence to human erythrocytes. The remaining radioactivity on the washed erythrocytes was determined after various incubation times and was expressed as the percentage of total radioactivity added. The results represent a typical experiment out of 4.
**TABLE 1. Effects of sialoglycoproteins and asialoglycophorin on adherence of *U. urealyticum* to human erythrocytes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycophorin</td>
<td>52 ± 20</td>
</tr>
<tr>
<td>Asialoglycophorin</td>
<td>0</td>
</tr>
<tr>
<td>α-Acid glycoprotein</td>
<td>29 ± 10</td>
</tr>
<tr>
<td>Fetuin</td>
<td>8 ± 6</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Buffer (control)</td>
<td>0</td>
</tr>
</tbody>
</table>

*α*-Acid glycoprotein, asialoglycophorin, fetuin, or glycophorin (0.1 mg/ml) was added to the suspension of *U. urealyticum* (serotype 8) prior to the erythrocytes in adherence experiments. In control experiments, bovine serum albumin or buffer was added.

Results are expressed as percentage inhibition compared with controls with buffer (5.5 to 7% adherence). Each value is the mean ± standard error of eight experiments.

The binding assay inhibited adherence by 53% ± 10%, while dextran (*M*~w~ ~ 500,000) inhibited adherence by 16% ± 6%.

The adherence of *U. urealyticum* seems to be of a complex nature, since it is mediated by proteinaceous and other surface components that recognize sialyl residues and/or sulfated compounds. These findings suggest that *U. urealyticum* possess adherence mechanisms resembling those found in other pathogenic mycoplasmas, such as *M. pneumoniae*, *M. gallisepticum*, and *M. pulmonis* (5, 6, 9, 12, 13, 15).

**REFERENCES**