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Use of the Transmission Electron Microscopy for Examination of Biofilms Structure

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Combination of transmission electron microscopy technics with colloidal gold labeled lectin staining allowed investigating glycoconjugates in biofilms of the corrosion relevant bacteria. Lectins with the same specificity were found to express different affinity to the compounds of the bacterial biofilms. LBA lectin showed the highest binding capacity to N-acetyl-D-galactosamine in *Desulfovibrio* sp. 10 and *Bacillus subtilis* 36 biofilms. For the assessment of N-acetyl-D-glucosamine presence in *Bacillus subtilis* 36 and *Pseudomonas aeruginosa* 27 biofilms WGA lectin was the most efficient. Staining with lectins labeled with the colloidal gold was proved to be a promising express technique for the investigations of glycoconjugate distribution and localization in bacterial biofilms.

Keywords: Transmission electron microscopy (TEM), Biofilm, Lectins, EPS.

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1. INTRODUCTION

In modern scientific world polyphasic analysis are becoming more wide-spread, allowing the comparison of the results obtained from different techniques to deliver detailed or comprehensive results. One of the approaches in the examination of bacterial cells surfaces are application of the both methods biochemical and electron microscopy with the labeled lectins.

Labeled lectins are widely used in the studies of the microbial cultures for cell surface structures probing, bacterial capsules investigation [3, 5], as well as for localization of the glycoconjugates on the cell surface [4]. The ability of lectins to express strong affinity to the oligosaccharides of the fully consistent structure allows examination of the certain glycoconjugates produced by the bacteria during the biofilm formation.

The aim of the study is to investigate the possibility to use lectins labeled with colloidal gold in combination with transmission electron microscopy in order to detect glycoconjugants in the biofilm formed by corrosion relevant bacteria on the steel surface.

2. METODS

Objects of the study. In our study bacteria isolated earlier from the corrosive sulfidogenic microbial community were used: *Desulfovibrio* sp. 10, *Bacillus subtilis* 36, *Pseudomonas aeruginosa* 27 [1].

Commercial preparations of the lectins with colloidal gold («Lectintest», Lvov, Ukraine) were used for biofilm labelling. Lectins were supplemented in the sol form, the size of the colloidal gold particles was 8-12 nm, lectin concentration was $A_{520} = 5.0 \pm 0.2$.

Biofilms samples for transmission electron microscopy were obtained by the replica method. Examination of lectin labled bacterial biofilms was performed using transmission electron microscope JEM-1400 («JEOL», Japan), under magnification 8000-12000. Minimally 20 grid meshes were viewed by randomization method. Number of the colloidal gold particles bounded with the biofilm glycoconjugates were counted in the field area 1 μm^2 using the program *Image J* ver. 143u ().

Statistical analysis was performed by calculating standard deviation of the colloidal gold particle number in the field using *MSExel 2010* software.

3. RESULTS AND DISCUSSION

Lectin specific binding capacity to the various cardohydrates allows to use them in the study of the features, structures and localization of the glycopolymers, as well as for identification of biological objects. However, according to manufacturer instruction, standard lectins were tested only with several carbohydrates. Therefore, we decided to examine the specificity of the commercially available lectins to the carbohydrates, produced in the biofilm formed by the corrosion-relevant bacteria.

Using the transmission electron microscopy technique we observed that lectin binding with carbohydrates occurred directly in the exopolymeric biofilm matrix and on the bacterial cell surfaces. Colloidal gold particles were detected in both cases. Colloidal gold labeled lectins were visualized on the images of the biofilm replicas as small round electron-dense particles with the size of 8-12 nm (Fig. 1).

Visualization of the LBA lectin bound to N-acetyl-D-galactosamine, synthesized by the biofilm bacteria is shown on the Fig. 1. On the region of *Desulfovibrio* sp. 10 biofilm replica image, marked with the red frame (Fig. 1a) and magnified (Fig. 1b), localization of colloidal gold labled LBA lectin is visible directly on the bacterial cell surface as well and in the biofilm matrix.

For the detection of the N-acetyl-D-galactosamine in the biofilm phaseolus lectin LBA and soybean lectin SBA were used (see Table. 1). In *Desulfovibrio* sp. 10 and *Bacillus subtilis* 36 biofilms lectin binding with glycoconjugants was detected due to the evidence of the

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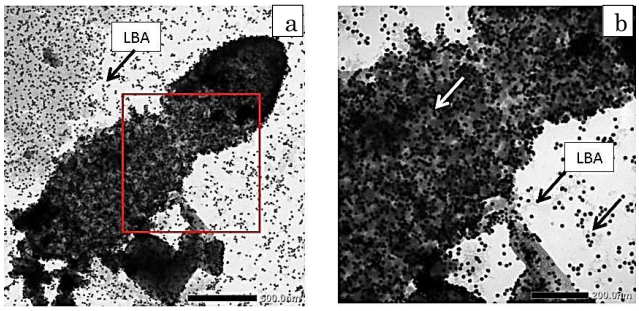

Fig. 1 – Lectin-binding to the glycoconjugants of *Desufovibrio* sp. 10 biofilm. Bar length a) – 500 nm, b) – 200 nm

colloidal gold particles presence. The numbers of LBA lectin particles binded to the N-acetyl-D-galactosamyne in the *Desulfovibrio* sp. 10 and *Bacillus subtilis* 36 biofilm were found to be 3.8 and 6 times more than the number of SBA lectin, respectively, suggesting that LBA lectin was more useful for examining biofilms of the bacteria studied.

In *Pseudomonas aeruginosa* 27 biofilm the numbers of the colloidal gold particles did not depend on the type of lectin and were almost equal for SBA and LBA lectins, specific for N-acetyl-D-galactosamine. Thus, for this strain detection of N-acetyl-D-galactosamine is equally efficient with both lectins.

For the detection of N-acetyl-D-glucosamine potatoe lectin (STA) and wheat lectin (WGA) were used. According to the results better binding to the *Desulfovibrio* sp. 10 biofilm was observed for STA lectin, and for *Bacillus subtilis* 36 и *Pseudomonas aeruginosa* 27 biofilms WGA lectin was more efficient.

Neutral carbohydrates such as D-mannose, D-glucose derivatives were detected using the lentil LSA, pea PSA lectins and concanavaline A (Con A). The highest number of the gold particles in all the biofilms examined was observed in case of PSA lectin staining. LSA lectin and concanavaline A slightly bound to D-mannose and D-glucose containing carbohydrates. Thus, the numbers of the colloidal gold labled particles of PSA lectin bound in the *Desulfovibrio* sp. 10 biofilm were 6.5 and 10.5 times more, than in case of LSA lectin and Con A respectively.

Thus, our investigations evidenced that lectins with the same glycoconjugate specificity bonded with carbohydrates containing in the corrosion relevant biofilms at different rates.

Table 1 – Lectin binding with glycoconjugants in the biofilms

Biofilm formed by bacteria	Lectin						
	LBA	SBA	STA	WGA	LSA	PSA	Con A
	Specificity						
	N-acetyl-D-galactosamine		N-acetyl-D-glucosamine		D-glucose, D-mannose		
*Desulfovibrio sp.*10	178 ± 15*	4717	109 ± 30	69 ± 32	21 ± 7	136 ± 30	13 ± 2
Bacillus subtilis 36	384 ± 33	65 ± 15	24 ± 7	100 ± 7	22 ± 6	51 ± 20	32 ± 12
Pseudomonas aeruginosa 27	53 ± 24	47 ± 18	46 ± 16	132 ± 11	15 ± 2	20 ± 11	11 ± 5

* – quantity of the colloidal gold parts per μm^2

The results obtained may be considered as the consequences of the lectin properties or target carbohydrate structure. Previous research [2, 7] showed that interaction of lectins with carbohydrates depended on their structures e.g. on the conformation of the pyranose and furanose forms of the glycoconjugate residues, contained in the carbohydrate part of the biofilm exopolymeric matrix. Lectin binding also depends on configuration of the carbohydrate (D- or L-form of the pyranose ring), from α- or β-position of the glycosidic bond at the C_1-position in monosaccharide. Consequently, spatial specificity, presence or absence of coordinate and hydrogen bonds, as well as hydrophobic and Van der Vaals forces should be considered as the most significant factors of the lectins binding with the carbohydrates.

Therefore we consider that while selecting lectins for biofilm glycoconjugates visualization carbohydrate, not only specificity should be taken into account, but also the data of biochemical investigations of the biofilm exopolymers and peculiarities of the bacterial cultures.

Basing on the information on the specificity of the lectins labeled with the colloidal gold, neutral carbohydrates (D-glucose, D-mannose) and animosaccharides (N-acetyl-D-glucosamine and N-acetyl-D-galactoseamine) were visualized in the bacterial biolifms. The same results were obtained with the use of fluorescent probes in lectin-binding analysis [8].

4. CONCLUSIONS

Thus, comparing the previous results of the biochemical analysis of the exopolymers, synthesized by the bacteria [6] with the data obtained from lectin-binding analysis we can conclude that the use of the lectins labeled with the colloidal gold is possible for the preliminary detection and localization of the carbohydrates in the biofilm matrix composition.

At the same time, in contrast to biochemical analysis, lectin-binding analysis allow to examine fully hydrated (native) biofilm matrixes in order to evaluate the presence of the compounds without extracting individual carbohydrates. Thus, lectin-binding analysis can be used in *situ*, for examining the glycoconjugates distribution in the bacterial biofilms.

Usage of the glycoconjugates specificity of the lectins labeled with the colloidal gold for the exanimation of the carbohydrates, synthesized by the bacteria, is the most suitable, accessible and fast conditional test-system, although this approach does not allow comprehensive characterization of the biofilm composition.

In this aspect, available lectins can be considered as valuable tools for the assessment of glycoconjugants

distribution in complex biofilm systems. Lectin-binding analysis can be also useas an express-method for the exopolymeric matrix composition evaluation in the fully hydrated and complex biofilm systems.

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