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Receptors for Endogenous and Heterogenous Hydroxamate Siderophores in *Staphylococcus aureus* B 47[1]

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A b s t r a c t

In *Staphylococcus aureus* B47 grown in iron-restricted medium, six new, iron-regulated proteins occurred in cytoplasmic membrane. Protein of 14kDa has bound two complexes of iron: Fe(III)-staphylobactin and Fe(III)-acinetoferrin. Complexes of Fe(III)-ferrichrome and Fe(III)-rhodotorulic acid were not bound to any of new membrane protein. Iron of Fe(III)-staphylobactin and Fe(III)-acinetoferrin complexes was transported into the cells.

K e y w o r d s: *Staphylococcus* sp., siderophores, iron regulated proteins.

Introduction

Microorganisms produce siderophores, high affinity iron chelating molecules, that solubilize Fe(III) and present it to the bacterial surface, where the complex may by transported across the bacterial envelope so that the iron can be used for biological processes.

Staphylococcus aureus may synthesize siderophores belonging to different chemical classes. Staphylobactin is a hydroxamate class chelator (Lisiecki and Mikucki, 1996; Lisiecki *et al.*, 1994; Lisiecki *et al.*, 2001), aureochelin belongs to the catecholate class (Courcol *et al.*, 1997) and staphyloferrin A and B have been classified as aminohydroxypolycarboxylic acids (Drechsel *et al*., 1993; Konetschny-Rapp *et al*., 1990). Heterogenous siderophores-bacterial and fungal, utilized by staphylococci as a source of iron, may be hydroxamate chelators-acinetoferrin, aerobactin, schizokinen, rhodotorulic acid, ferrioxamine B, ferrichrome and catecholates ones – N-(2,3-dihydroxybenzoyl)-glycine, N-(2,3-dihydroxybenzoyl)-L-serine, 2,3-dihydroxybenzoic acid and enterobactin (Lisiecki and Mikucki, 1996; Sebulsky *et al*., 2000).

The transport system of Fe(III)-siderophore complex in bacterial cell is composed of a receptor, binding protein dependent transport (BPT) and permease complex (Clarke *et al*., 2001). In *S.aureus* several proteins involved in Fe(III)-hydroxamate complexes uptake have been detected. FhuD1 and FhuD2 proteins form binding proteins transport system. Two proteins – Fhu B and Fhu G are a complex of permease and the third one Fhu C – ATP binding protein with traffic ATP-ase activity (Cabrera *et al*., 2001; Sebulsky and Heinrichs, 2001; Sebulsky *et al*., 2000).

The aim of this study was to detect and localize siderophore receptors in the cell and estimate their specificity for Fe(III)-hydroxamate siderophore complexes.

Abbreviations: AB – p-azidobenzoyl analog of siderophore, AC – acinetoferrin, FC – ferrichrome, RA – rhodotorulic acid, SB – staphylobactin.

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Experimental

Materials and Methods

Bacterial strain. *Staphylococcus aureus* B47 isolated from the human nasopharynx was used. The strain was identified with API-STAPH (bioMerieaux) and stored at –70°C in 50% glycerol solution in 3.7% Brain Heart Infusion Broth (Difco).

Siderophores. Acinetoferrin AC (gift from Professor Shigeo Yamamoto, University of Yokohama), ferrichrome FC (Sigma), rhodotorulic acid RA (Sigma) and staphylobactin SB, extracted and purified in our laboratory were used.

Medium and growth conditions. The chemically defined medium CDM of pH 7.2, sterilized with filtration through a membrane filter of 0.22 mm pore width (Milipore) was used (Lisiecki *et al*., 1994). The iron content was reduced using polyaminocarboxyl resin Chelex 100 (200–400 mesh) (BioRad). The bacteria were grown in iron-deficient CDM medium (CDM-Chelex) and with excess of iron content (1×10^{-4}M, CDM-Chelex +Fe) in the form of $FeSO_4 \times 7H_2O$.

A portion of an appropriate medium was inoculated with 18 hour, iron starved culture in CDM-Chelex medium constituting 5% of total volume. The culture was incubated for 24 hours in temperature of 37ºC with constant shaking (120 strokes/min). The culture was centrifuged (1600×g, 15min, 4ºC), supernatant was assayed for siderophore content and cells were subjected to the process of protoplast formation and membrane preparation.

Suspensions optical density. The optical density of suspension and cultures was measured in UV/Vis Cary 1 (Varian) spectrophotometer at 540 nm.

Viable count. Viable count was estimated by using serial dilutions in buffered 0.155 M NaCl, pH 7.2 (Biomed) and standard plate method on 4% Tripticase Soy Agar (Difco).

Protoplast formation and cytoplasmic membrane preparation. Protoplasts were prepared using lysostaphin (Sigma) and lysozyme (Serva) according to the method of Theodore *et al*. (1971) as modified by Lindberg (1981). The protoplast lysate in 0.01 M Tris – HCl (pH 7.2) buffer containing membranes and cytoplasm was pre-centrifuged (3000×g, 15 min, 4°C), the pellet discarded and the supernatant was centrifuged 100 000×g, 60 min, 9°C (Beckman, L8-70M). The collected membranes were washed three times with deionised water and lyophilized.

Siderophores determination. Total activity of siderophores was assayed by the Schwyn and Neilands (1987) method with Chrome Azurol S. Hydroxamate siderophore-staphylobactin was assayed according to Csaky (1948) and Emery and Neilands methods (1962). The results were expressed as μg of the desferrioxamine mesylate (Desferal, Ciba-Geigy) and calculated for millilitre of culture supernatant.

Staphylobactin isolation and purification. Siderophore was isolated and purified according to the method of Okuyo *et al*. (1994) modified by Lisiecki *et al*. (1994). The bioactivity of the siderophore during purification was assayed according to the method of Reissbrodt and Rabsch (1988). The culture supernatant of *S.aureus* B47 strain was adjusted to pH 6 with 60% citric acid. An 80 g of resin Amberlite XAD 7 (Aldrich) was added to 4000 ml of supernatant and suspension was shaken slowly for 1 hour. The suspension was filtered and the pellet was washed three times with 1000 ml of distilled water. The resin was eluted with 200 ml of methanol and incubated at room temperature for 30 min, filtered and then washed with 100 ml of methanol. This procedure was repeated twice more. The eluate was evaporated at 30°C and the residue was dissolved in 100 ml of water. The solution adjusted to pH 2.0 with solid citric acid was extracted three times with 200 ml of ethyl acetate. After washing three times with 0.1 M sodium citrate, pH 5.5, the organic layer was evaporated to give a crude siderophore fraction. It was dissolved in 5 ml of methanol and the insoluble materials were removed by centrifugation. The resulting solution was subjected to preparative paper chromatography on Whatman No 3 paper in mixture of solvents n-butanol-acetic acid-water (63:25:12). A strip of paper was taken and sprayed with Schwyn and Neilands (1987) reagent containing Chrom Azurol S to locate the siderophore. The colored spot of Rf = 0.2 exhibited siderophore activity. Corresponding regions of the remaining papers were excised and extracted with methanol-water (1:1) solution. After evaporation of the solvent, the residue was deferrated 1.0 M KOH, centrifuged (9000×g, 20°C, 10 min) and lyophilized. This material was positive in the plate bioassay with *S. aureus* B47 strain and the Csaky (1948), Emery and Neilands (1962) and Schwyn and Neilands (1987) tests.

Photoaffinity labels of siderophores. Photoaffinity labelling of siderophores was performed according to Nelson *et al*. (1992) methods. Siderophores were referrated with $^{59}FeCl_3$: 500 μg of siderophore was first dissolved in 50 μL of methanol to which 50 μCi $^{59}FeCl_3$ (NEN) and 100 μL of dimethylformamide (Sigma) were added and, to AC solutions only, 100 μL of triethylamine (Ubichem). The reaction mixtures were gently shaken to aid dissolution. The N-hydroxysuccinimidyl-4-azidobenzoate (Sigma) was then dissolved in these solutions and the reaction was allowed to proceed for 24 hours at 25°C. All preparations involving photoreactive compounds were done under very low light conditions. The particular solutions contained photoreactive p-azidobenzoyl analogues of the siderophores labeled with ^{59}Fe(III).

Photolabelling of the membrane proteins. Lyophilized membranes were diluted with HEPES buffer of pH 7.2 and 20 μL of each sample was mixed with 5 μM labelled siderophore solution. The mixture was chilled in ice-water bath for 10 min and photolysed for 2 min using UV lamp. The membranes were pelleted and washed three times with HEPES buffer by centrifugation (100 000×g, 60 min, 9°C).

Electrophoresis. Membrane proteins were separated electrophoretically according to Laemmli (1970) method in Multiphor 2 (Pharmacia Biotech) apparatus using 12.5% Excel Gel SDS (Pharmacia Biotech) and buffered strips (Pharmacia Biotech). The set of standard proteins Low Molecular Weight Calibration Kit (Pharmacia) 14.4 to 94 kDa was used. The investigated samples contained about 15 μg of protein in 10 μL. All the samples and the standard proteins were boiling for 2 min in a 100ºC water bath.

Non-radioactive gels were stained with Coomassie Blue (Merck). Gels of the labelled siderophores were subjected to autoradiography using Kodak X-Omar film for 24 hours. Following autoradiography these gels were stained with Coomassie blue and photographed. The analysis of electrophoretic separation was performed with computer programme Image Master (Pharmacia Biotech).

Time-dependent uptake of radioactive iron sources. *S. aureus* B47 strain was grown in CDM-Chelex medium. The cells were harvested after 24 hours of incubation and washed three times with buffered 0.155 M NaCl, pH 7.2 (Biomed) by centrifuga-

tion (1600×g, 20 min., 4°C). The pellet was suspended in CDM-Chelex medium to give a final cell concentration of about 3×10^8 cfu ml^{-1}. Suspension was then divided into 200 µL portions. 10 µL of radioactive iron sources containing 10 µM ^{59}Fe ferrated siderophore was added to series of 7 tubes. At various intervals, after 5, 10, 15, 20, 25, 30 and 40 minutes one tube was withdrawn. The suspension was filtered through a membrane filter of 0.22 mm pore (Milipore), washed three times with buffered saline (Biomed) pH 7.2. The radioactivity of the cells was measured in a Wallac 1470 Wizard gamma counter.

Blocking of receptors and uptake of radioactive iron sources. *S. aureus* B47 strain was grown in CDM-Chelex medium. The cells were harvested after 24 hours of incubation, washed three times with buffered 0.155 M NaCl, pH 7.2 (Biomed) by centrifugation (1600×g, 20 min, 4°C) and resuspended in appropriate volume of fresh CDM-Chelex medium. The density of suspension corresponded to 3×10^8 cfu ml^{-1}. To 1 ml of suspension, 10 µL solution containing 10 µM photoreactive p-azidobenzoyl analogues of siderophores saturated with Fe(III) was added. The suspension was poured to Petri dishes and immediately, using UV lamp, photolysed for 2 minutes. After photolysis the suspension was centrifuged (3000×g, 5 min, 4°C), washed three times with HEPES buffer, pH 7.2, suspended in the initial volume of CDM-Chelex medium and viable count (cfu ml^{-1}) was estimated. 10 µL of solution containing 10 µM labeled ^{59}Fe (III) siderophore was added per milliliter of suspension. After 25 minutes of incubation the suspension was filtered through 0.22 µm membrane filter (Milipore) and washed three times with buffered 0.155 M NaCl, pH 7.2 (Biomed). The radioactivity of cells was measured in a Wallac 1470 Wizard gamma counter.

Analytical methods. Protein was assayed with Lowry *et al*. (1951) method. Iron content was determined with Gadia and Mehra method (1977). Statistical analysis was performed with the Statistica PL computer programme (StatSoft).

Results

The iron content in CDM-Chelex medium varied in range of 9.5×10^{-7} to 1.2×10^{-6} M. The detectable siderophores activity in CDM-Chelex medium was found in the 5th–6th hour of incubation: in the middle of the exponential growth phase. The highest activity has occurred in the 15th–22th hour, in the stationary growth phase. The siderophores were not produced in CDM-Chelex + Fe medium.

The investigated strain harvested from CDM-Chelex and CDM-Chelex + Fe media, after 25 hours of incubation was subjected to protoplast formation. In a typical experiment, after 60 minutes the optical density of the suspension was maintained at the level equal to 32% of the initial value and over 90% of gram-negative cells were found.

The cytoplasmic membrane protein profiles of *S. aureus* B47 strain grown in CDM-Chelex and CDM-Chelex + Fe media were compared with a computer programme Image Master. This technique revealed six new band-iron regulated proteins that were expressed only in cytoplasm membranes from cells grown under iron limitation in CDM-Chelex medium. They corresponded to proteins with apparent molecular masses: 14 kDa with Rf = 0.92 which might be a complex of two peptides and at the region of 96–43 kDa with molecular masses of 96, 88, 80, 69, 43 kDa and Rf values of 0.25, 0.29, 0.34, 0,38 and 0.54, respectively (Fig. 1; lane 1A).

Searching for proteins which might be siderophore receptors, autoradiograms of gels containing cytoplasmic membrane proteins of cells grown under iron deficiency (CDM-Chelex) and labelled by p-azidobenzoyl complex [^{59}Fe(III)]AB were analysed and referred to electrophoretic separations of cytoplasmic membrane proteins. Then they were compared with electrophoregrams of the membranes of cells

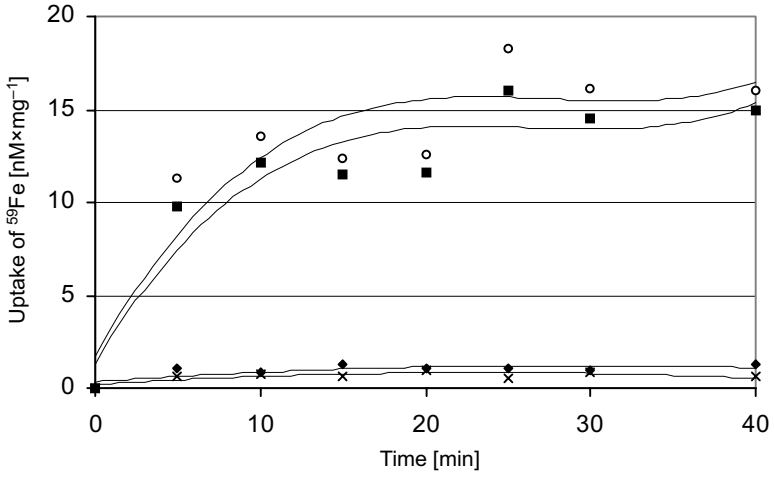

Fig. 2. Iron uptake by *S. aureus* B47 from [^{59}Fe(III)]-staphylobactin -○-, [^{59}Fe(III)]-acinetoferrin -■-, [^{59}Fe(III)]-ferrichrome-x-, [^{59}Fe(III)]-rhodotorulic acid -◆- complexes.

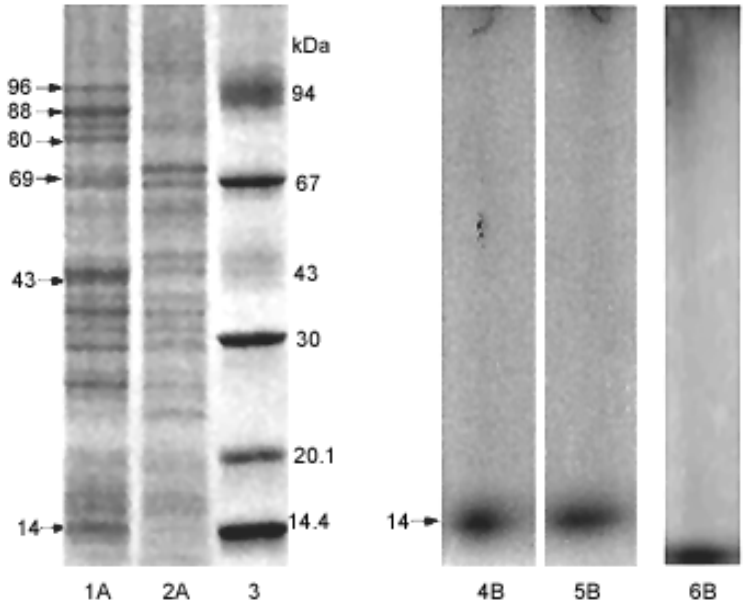

Fig.1. SDS-PAGE profiles of cytoplasmic membrane proteins of *S. aureus* grown in CDM-Chelex medium (lane1A) and CDM-Chelex+Fe medium (lane 2A). Autoradiograms of membrane proteins (lane1A), labelled with [^{59}Fe(III)]-staphylobactin (lane 4B), [^{59}Fe(III)]-acinetoferrin (lane 5B), [^{59}Fe(III)]-rhodotorulic acid (lane 6B) complexes

grown in the presence of iron excess (CDM-Chelex+Fe) (Fig.1; lanes B). Acinetoferrin complex ([^{59}Fe(III)]ABAC) was bound to the cytoplasmic membrane protein with the molecular mass of 14 kDa and Rf of 0.92, constituting 4.3% of total membrane proteins. Staphylobactin complex ([^{59}Fe(III)]ABSB) was also bound to the protein with the molecular mass of 14 kDa. Rhodotorulic acid complex ([^{59}Fe(III)]ABRA) has not bound any of new iron-regulated proteins, because a signal of Rf=1 in autoradiograms did not correspond to any on electrophoregrams. The Rf value of this signal has shown that it occurs in the region of low-molecular membrane proteins. Thus, the movement in electric field was determined by binding to low-molecular protein that, however, could have a non-specific character. Ferrichrome complex ([^{59}Fe(III)]ABFC) did not occur on autoradiograms, thus this siderophore was not bound to any of membrane proteins. The control electrophoresis of protein-unbound ^{59}Fe(III)AB-siderophore complexes revealed that they do not move in the electric field, remaining on the autoradiograms at the start line. The control of viable count of cells after the photolysis showed that in the suspension containing about 3.6×10^8 cfu ml^{-1} an average 70% of cells remained.

To prove participation of the studied siderophores and their receptors in iron Fe(III) transport into the cell, kinetics of iron [^{59}Fe(III)]-siderophore complexes uptake by resting cells was investigated (Fig. 2). Complexes of [^{59}Fe(III)]AC and [^{59}Fe(III)]SB after binding by the receptor were transported into the cell. Following the initial phase of fast uptake of [^{59}Fe(III)]AC complex, until 25 min of incubation, the linear phase of slow iron uptake occurred. After 25 min the cells contained 9% initial dose of isotope. After 30 and 40 min the isotope content in cells was 8.1% and 10.2% of isotope added in the t_o time of the experiment, respectively. When staphylobactin was ligand of ^{59}Fe(III), after 25 min of incubation the amount of assimilated isotope was 10.3% of the initial dose. After 30 min it increased slightly and at the end of the observation it reached 11.5% of the initial isotope dose.

The examination of ^{59}Fe(III) iron uptake from ferrichrome ([^{59}Fe(III)]FC) and rhodotorulic acid ([^{59}Fe(III)]RA) was a control because these fungal chelators were not heterogenous siderophores for *S. aureus* B47 strain. Flat uptake curves without maximum reflected very small amounts of the isotope, which were quite different in comparison with the iron uptake from the acinetoferrin and staphylobactin complexes bound to receptors.

When 14 kDa receptor was blocked by p-azidobenzoyl analogue of Fe(III)-staphylobactin (Fe(III)ABSB), after 25 min of incubation cells of *S. aureus* B47 strain contained only 0.9% and 0.8% of the initial isotope doses of [^{59}Fe(III)]SB and [^{59}Fe(III)]AC respectively per mg of dry bacteria mass. After blocking this receptor by p-azidobenzoyl analogue of Fe(III)-acinetoferrin complex (Fe(III)ABAC) the cells contained 0.8% and 0.7% of the initial doses of [^{59}Fe(III)]AC and [^{59}Fe(III)]SB per mg, respectively (Table I).

Table I
Iron uptake from [^{59}Fe(III)]-staphylobactin and [^{59}Fe(III)]-acinetoferrin complexes by *S. aureus* B47 cells with blocked and unblocked 14 kDa receptor

Unblocked receptor						Blocked receptor											
S. aureus B47																	
						Fe(III)-AB**)staphylobactin						Fe(III)-AB**)acinetoferrin					
^{59} Fe(III) initial dose																	
[^{59}Fe(III)]-staphylobactin			[^{59}Fe(III)]-acinetoferrin			[^{59}Fe(III)]-staphylobactin			[^{59}Fe(III)]-acinetoferrin			[^{59}Fe(III)]-acinetoferrin			[^{59}Fe(III)]-staphylobactin		
cpm*) mg^{-1}***)	nM mg^{-1}	% initial dose	cpm*) mg^{-1}***)	nM mg^{-1}	% initial dose	cpm*) mg^{-1}***)	nM mg^{-1}	% initial dose	cpm*) mg^{-1}***)	nM mg^{-1}	% initial dose	cpm*) mg^{-1}***)	nM mg^{-1}	% initial dose	cpm*) mg^{-1}***)	nM mg^{-1}	% initial dose
12192 ±1207	178.3	100	13624 ±1720	178.3	100	13106 ±1700	178.3	100	13106 ±1700	178.3	100	13106 ±1700	178.3	100	13106 ±1700	178.3	100
Iron uptake																	
1251 ±91	18.29	10.3	1223 ±273	16.01	9.0	114 ±16	1.55	0.9	106 ±22	1.44	0.8	101 ±24	1.39	0.8	91 ±15	1.24	0.7

*) cpm, counts per minute; **) AB, p-azydobenzoyl analogue; ***) mg dry weight of bacteria

The statistical analysis has proved that differences between mean values of iron uptake in time from Fe(III)AC, Fe(III)SB complexes and Fe(III)FC, Fe(III)RA complexes were significant ($p<0.05$). The differences between mean values of iron uptake from Fe(III)AC, Fe(III)SB complexes by cells with blocked and unblocked receptors were also significant ($p<0.05$). All these results were confirmed with the LSD test (least significant differences) ($p<0.05$).

Discussion

Staphylobactin belonging to the citrate-based hydroxamate class chelators has been found to be the endogenous siderophore of *S. aureus* B47 strain (Lisiecki *et al*., 1994; Lisiecki and Mikucki, 1996; Lisiecki *et al*., 2001). Heterogenous siderophores utilised by this strain belonged to the catecholate class chelators – N-(2,3-dihydroxybenzoyl)-glycine and N-(2,3-dihydroxybenzoyl)-L-serine as well as to chelators of the hydroxamate class of citric acid derivatives-aerobactin, schizokinen and acinetoferrin (Lisiecki *et al*., 2001).

Most of bacterial siderophore systems are subjected to derepression at iron Fe(III) concentrations of 10^{-7} M but the concentration of 10^{-6} optimally regulates their expression (Hider, 1984). Iron concentrations in CDM-Chelex medium were within the range between 9.5×10^{-7} M and 1.2×10^{-6} M fulfilling condition of derepression. Under iron-limitation conditions *S. aureus* B47 strain has demonstrated simultaneous expression of siderophores and receptor proteins.

There is no reports about iron-regulated proteins in *S. aureus*, which could be receptors for Fe(III)-siderophore complexes. In cell lysates of blood-isolated *S. aureus*, at least one protein was detected in the region of 36–39 kDa, occurrence of which correlated with siderophores release (Lindsay and Riley, 1994). Proteins with higher molecular mass were also found (Courcol *et al*., 1997; Trivier *et al*., 1995). The presence of such proteins in the region of 81–17 kDa was observed in cell lysates from two *S. aureus* strains, DES and DAU, isolated from cases of mucoviscidosis. One of them, 81 kDa, was repressed in the presence of iron excess. Two other proteins – 23 and 17 kDa occuring together with siderophores did not undergo repression (Trivier *et al*., 1995). The expression of three proteins with masses of 120, 88 and 57 kDa occurred exclusively under iron-deficient conditions (Courcol *et al*., 1997).

In the isolated cytoplasmic membrane of *S. aureus* B47 strain grown in iron-deficient CDM-Chelex medium there was always the same profile of new, iron regulated proteins with masses of 96, 88, 80, 69, 43 and 14 kDa. The 14 kDa protein was formed with two closely localized bands in the electrophoregram. These iron-regulated proteins were probably anchored in the cytoplasmic membrane as cell fractionation by lysostaphin did not remove them, similarly as in *S. epidermidis* ABC transporter lipoprotein (Cockayne *et al*., 1998). The protein with the mass of 43 kDa was identified as the ferrioxamine B receptor (Wysocki *et al*., 2003). The second receptor protein – 14 kDa bound two chelators *i.e.* staphylobactin and acinetoferrin.

There were no receptors for ferrichrome and rhodotorulic acid. Subsequent blocking 14 kDa receptor by Fe(III)-AC and Fe(III)-SB complexes gave the evidence that they were bound to only one of these two proteins forming in the electrophoregrams the band of 14 kDa mass. It also demonstrated that this receptor protein participated in Fe(III)-siderophore complex transport into the cell. So, this protein met the criteria for receptors function. It not only recognised and bound Fe(III)-siderophore complex but also took part in its transport to the cells presenting it to the subsequent component of the siderophore system.

In *E. coli* outer membrane protein Fhu E is the receptor for two siderophores also. It has bound coprogen and rhodotorulic acid, structurally related to coprogen, both linear fungal chelators (Hantke, 1983). Another protein – Cir was capable of transporting iron complexes very similar structurally siderophores: dihydrobenzoic acid-enterobactin precursor and dihydrobenzoylserine-enterobactin breakdown product (Hantke, 1990).

In order to use hydroxamate siderophores by *S. aureus* an operon *fhuCBG*, another two genes – *fhuD1* and *fhuD2* are required (Cabrera *et al*., 2001; Sebulsky and Heinrichs, 2001; Sebulsky *et al*., 2000). Genes *fhuD1* and *fhuD2* encode proteins belonging to binding protein dependent transport system (BPT) (Sebulsky and Heinrichs, 2001). Two homologous proteins of this system FhuD1 and FhuD2, have overlaping but different specificity, lower than receptor proteins (Sebulsky and Heinrichs, 2001). The FhuD1 co-operates with linear hydroxamate siderophores ferrichrome and ferrioxamine B while FhuD2 co-operates with ferrichrome, ferrioxamine B and coprogen, also linear siderophores and aerobactin, symmetric derivative of citric acid. Affinity of various Fe(III)-hydroxamate siderophore complexes to FhuD1 and FhuD2 proteins is diverse and depends on the environmental conditions (Sebulsky and Heinrichs, 2001). The FhuD2 protein transports aerobactin being a symmetric derivative of citric acid (Sebulsky and Heinrichs, 2001). Staphylobactin, and acinetoferrin also have such a structure. Purified staphylobactin contained citric acid and amino acids-glycine, alanine and leucine. The initially proposed its structure was citric acid as central backbone linked to two lateral chains of amino acids (Lisiecki and Mikucki, 1996). The backbone of acinetoferrin is citric acid also, which is linked to two lateral chains of 1,3-diaminopropane (Okuyo *et al*., 1994). In bioassays these two siderophores, like aerobactin, have promoted growth of two indicators strain – *E. coli* LG 1522 and *A. flavescens* JG-9 requiring for growth different hydroxamate chelators, proving that they have belonged to hydroxamate siderophores group-symmetric derivatives of citric acid (Reissbrodt and Rabsch, 1988; Lisiecki and Mikucki, 1996). Thus, both siderophores are hydroxamate chelators-containing hydroxamic groups and additionally iron-binding ligands in form alpha-hydroxycarboxylate residues which coordinate iron Fe(III) (Winkelman and Drechsel, 1997). These siderophores can be thus transported into the cell by FhuD2 protein. The transport of these siderophores by the same BPT protein, and, especially, binding by the same receptor confirms structural similarity of staphylobactin, acinetoferrin and aerobactin.

The FhuD2 protein transports the adequate Fe(III)-siderophore complex to the subsequent transport link – Fhu CBG proteins encoded by the *fhuCBG* operone (Cabrera *et al*., 2001; Sebulsky *et al*., 2000). FhuC is ATP – binding protein with traffic ATP-ase activity, while FhuB and FhuG form the complex of transmembrane permease with a wide range of specificity, passing Fe(III)-siderophopre complexes across the cytoplasmic membrane and are necessary for hydroxamate siderophores uptake (Cabrera *et al*., 2001; Sebulsky *et al*., 2000). There is alack of one important link in this system iron assimilation – *i.e.* receptor. The recognition of the complex and binding to the membrane receptor is an essential prerequisite for transport of Fe(III)-siderophore complex into the cell. Acinetoferrin and staphylobactin were bound to 14 kDa protein receptor and transported with permease as [^{59}Fe(III)]AC and [^{59}Fe(III)]SB complexes were detected in *S. aureus* B47 strain cells. Ferrichrome and rhodotorulic acid for which *S. aureus* B47 strain did not have membrane receptors, were not transported into the cell. In the previous investigations it has been found that the growth of *S. aureus* B47 strain was promoted by its endogenous siderophore-staphylobactin and heterogenous one-acinetoferrin and was not promoted by heterogenous ones-ferrichrome and rhodotorulic acid (Lisiecki *et al*., 2001). The presented study allowed to prove that *S. aureus* B47 strain growth was the result of binding these siderophores to the receptor and stimulating growth transport Fe(III) into the cell.

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