1 Abstract

Pseudomonas aeruginosa is an ubiquitary and opportunistic gram negative bacterium. Patients who suffer from the autosomal-recessive cystic fibrosis have an infection with the antibiotics resistant bacterium, *Pseudomonas aeruginosa*, which leads to a lethal progression of the disease (Elkin and Geddes, 2003; Warneer, 1992). The adhesion to epithelial cells of the respiratory tract is deemed to be the initiating step in *Pseudomonas aeruginosa* infection. The D-galactose-specific lectin PA-I, as well as the L-fucose-specific lectin PA-II and the pili plays an important role in the adhesion. The GalNAc-Gal-specific adhesion function of the pili is located at the pilin subunits which assemble the pili. The main concern is the development of effective therapeutics. The employment of equal saccharides for the inhibition of the adhesion is limited by glycolysis which leads to a short half life of the saccharides, of a few minutes. To manage this problem it is necessary to transfer the characteristics of the saccharides to other molecules. The possibility to use peptides to mimic saccharides was the basis for the thesis. The aim of this thesis was the search for glyco-replica-peptides able to mimic saccharide-structures which play important roles in the adhesion of *Pseudomonas aeruginosa* to epithelial cells of the respiratory tract by *Random Peptide Phage Display Libraries*. The use of *Random Peptide Phage Display Libraries* affords the employment of peptide presenting phages with a variance of 10^8 to 10^{10}. The peptide displayed by the phage which is interacting with the target is easy to identify by the inserted sequences in the phage genome. The D-galactose-specific lectin PA-I, the L-fucose-specific lectin PA-I and a synthesized peptide of the pilin subunit, PAK-Pilin-Protein (128-144) KCTSDQDEQFIPKGCSK were chosen as targets for the isolation of possible glyco-replica-peptides. A commercial 12-mer *Random Peptide Phage Display Library* was used for each *in vitro* selection process, called *Biopanning*. The *Biopanning* with the D-galactose-specific lectin PA-I identified to specific binding phages with the sequence **SHLDPTLFPLYK**. These phages were found by specific elution with phenyl-β-D-galactopyranoside and also by an unspecific elution with glycine-HCl (pH 2,2). A consensus sequence of eight amino acids **PTLFPLYK** was identified. The binding of the phage with the sequence **SHLDPTLFPLYK** showed the highest affinity to the Lectin PA-I and was inhibited

by the phenyl-β-D-galactopyranoside, whereas phenyl-β-D-glucopyranoside used as control showed no inhibition.
The binding of the biotinylated *Pseudomonas aeruginosa* lectin PA-I to the adsorbed glycoprotein P1 was inhibited in a concentration dependant manner by Phenyl-β-D-galactopyranosid and by the identified peptide. By this way the mimic potential of the identified peptide was confirmed. The specificity of this sequence was approved by competition studies with the synthesized peptide sequence using the *surface plasmon resonance* method (BIACORE). In a functional *in vitro* test the binding of the of biotinylated bacteria *Pseudomonas aeruginosa* to A549 human lung carcinoma cells could be inhibited by the found D-galactose-replica-peptide. For the first time a D-galactose-replica-peptide was found that achieved the desired function.

For the L-fucose-specific lectin PA-II *Biopanning* was performed with specific L-fucose elution and repeated two times. The first *Biopanning* yielded 19 specific binding phages. The two sequences (**A**) **SSAWWSYWPPVA** (7 x) and (**B**) **SWPYSFWFPLEN** (5 x) were found with high frequency. The second *Biopanning* yielded 31 specific binding phages. The sequence (**C**) **ILANDLTAPGPR** (15 x) and (**D**) **AHRHPISFLSTL** (6 x) were found with high frequency. These four phages bound to the target L-fucose-specific lectin PA-II as well as to the D-galactose-specific lectin PA-I. The binding of the phages to the L-fucose-specific lectin PA-II could only be inhibited by very high concentrations of L-fucose. Further investigations of the specificity of the identified phages for the lectin PA-II binding were confined to the sequence (**A**) **SSAWWSYWPPVA** because of the highest ratio of hydrophobic amino acids. A higher affinity of hydrophobic ligands to the *Pseudomonas aeruginosa* lectin PA-II was described by Garber et al. (1987 and 1992). There were no differences for inhibition of the phage binding between the synthesized peptide **SSAWWSYWPPVA**C and by a scrambled peptide with an arbitrary order of the 12 amino acids. The peptide **HSVSNIRPMFPS**C from the *Biopanning* against the PAK-Pilin-Protein (128-144) used as control showed no inhibition. The specificity of the peptide sequence **SSAWWSYWPPVA**C was demonstrated on the basis of a BSA-conjugate. The BSA-conjugate of the arbitrary sequence and the control BSA-conjugate showed no inhibition of the phage binding. The peptide-**SSAWWSYWPPVA**C-BSA-conjugate showed an inhibition of 31 %.

With the non-biotinylated PAK-Pilin-Protein (128-144) the *Biopanning* yielded 18 specific binding phages. For the investigations of the specificity the most frequently identified sequence **HSVSNIRPMFPS** was used. The binding of the biotinylated PAK-Pilin-Protein (128-144) to the ganglioside asialo GM1 was inhibited by the synthesized peptide **SSAWWSYWPPVA**C and as well as by the peptide with an arbitrary order of the 12 amino acids. There were no significant differences between the two peptides. A control peptide showed no inhibition.

Three glyco-replica-peptides were isolated and identified which mimic the searched saccharide-ligands for inhibition of the bacterium *Pseudomonas aeruginosa*.

- **S H L D P T L F P L Y K** as D-galactose-replica-peptide
- **S S A W W S Y W P P V A** as L-fucose-replica-peptide
- **H S V S N I R P M F P S** as β-D-GalNAc-(1-4)-β-D-Gal-replica-peptide

Further investigations are necessary to employ these peptides to protect cystic fibrosis patient from infection with *Pseudomonas aeruginosa* and increase the life expectancy of the patients.