

## Protein component of *Pseudomonas aeruginosa* slime glycolipoprotein in relation to laboratory-induced resistance to gentamicin

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Isoelectric focusing of the slime glycolipoprotein of a gentamicin-sensitive *Pseudomonas aeruginosa* strain showed the presence of four protein bands at a pH range of 5.45-6. Two additional protein bands at about pH 5.62 and pH 5.3 appeared in the slime glycolipoprotein of a laboratory-induced gentamicin-resistant variant.

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L'électrofocalisation de la couche visqueuse de glycolipoprotéine d'une souche de *Pseudomonas aeruginosa* sensible à la gentamycine montre la présence de quatre bandes de protéines à des pH se situant entre 5.45-6.0. Chez un variant résistant à la gentamycine, cette résistance ayant été induite en laboratoire, deux bandes additionnelles sont apparues aux pH 5.62 et pH 5.3.

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Gentamicin-modifying enzyme negative resistant and susceptible strains of *Pseudomonas aeruginosa* show differential permeability to gentamicin (Bryan and van den Elzen 1977). Previous work on the character of *P. aeruginosa* in relation to laboratory-induced resistance to gentamicin has shown that the resistant progenies differed from the parent strain in lytic phage patterns, phage adsorption, and agglutination with specific anti-lipopolysaccharide sera (Dimitracopoulos et al. 1979).

The extracellular slime glycolipoprotein (GLP) of *P. aeruginosa* is considered 2-3 times more toxic than the lipopolysaccharide (LPS), has anti-phagocytic activity, is produced in vivo, and possesses receptorlike properties for phages (Bartell et al. 1971; Dimitracopoulos et al. 1974; Reese et al. 1974; Sensakovic and Bartell 1974). Therefore, it seemed interesting to study the chemical composition and the biological properties of the GLP extracted from a laboratory-induced resistant variant of *P. aeruginosa*. In this note we report our results on the protein component of the GLP subjected to isoelectric focusing.

The organism used, *P. aeruginosa* strain C2, was isolated from a clinical specimen. The minimal inhibitory concentration (MIC) of gentamicin was

found to be 0.4 µg/mL. A laboratory resistant variant grown in 250 µg/mL was produced from the sensitive wild strain by repeated passage in trypticase soy broth (Baltimore Biological Laboratories) containing increasing (twofold) levels of gentamicin and up to 250 µg/mL.

The GLP fraction was obtained from the extracellular slime layer of strain C2 and its resistant variant by a method described previously (Bartell et al. 1970; Sensakovic and Bartell 1974). Slime was extracted in 0.15 M NaCl from 18-h bacterial cultures grown on sheets of cellophane overlaying trypticase soy agar. The extract was precipitated with ethanol, clarified by centrifugation, and dialyzed against distilled water. The dialysate was centrifuged at 105 000 × g for 3 h and the supernatant fluid with the GLP fraction was lyophilized. The GLP was then filtered through gels and subjected to anion-exchange chromatography, eluting at a KCl molarity of 0.3-0.4. Purity and homogeneity were demonstrated by chromatography, sedimentation pattern, and immunodiffusion (Sensakovic and Bartell 1974). Lyophilized GLP was stored in vacuo at 4°C.

Isoelectric focusing was performed in 5% polyacrylamide gels with 2% Sevalyt, pH 5-8 (Serva) by using the method of Righetti and Dry-

dale (1971). At the end of electrofocusing one part of the gel was fixed for 12 h in 12.5% trichloroacetic acid and then stained for 3 h at 37°C in a solution containing methanol (75 mL), distilled water (186 mL), trichloroacetic acid (30 g), sulfosalicylic acid (9 g), and Coomassie brilliant blue R 250, to a final concentration of 0.1%. Destaining was performed by washing the gel in a solution of ethanol (250 mL), water (650 mL), and glacial acetic acid (80 mL) (Vesterberg 1971). Then, the gels were scanned with a Zeiss scanner recording the absorbance at 620 nm. The unstained part of the gel was cut into pieces which were eluted in 1 mL of distilled water for 1 h and the pH of each section was determined.

Figure 1 shows the scanning profiles of gels with the respective pH gradient. The slime GLP of the sensitive wild *P. aeruginosa* strain C2 shows the presence of four protein bands. They are numbered from the cathode 1, 2, 4, and 5, and appear in the range of pH 5.45–6. The slime GLP of the resistant variant shows the presence of two more protein bands, 3 and 6, at about pH 5.62 and pH 5.3, respectively.

Laboratory-derived resistant strains of *P. aeruginosa* appear different in their growth rates and virulence for mice (Weinstein 1973), whereas their resistance to gentamicin is accompanied by loss of their ability to grow at 42°C and of their receptor sites for phages (Dimitracopoulos et al. 1979). Besides, it has been reported that the amount of surface lipids in laboratory-induced resistant variants of *P. aeruginosa* is greater than that of the parent sensitive strains (Pechey and James 1973).

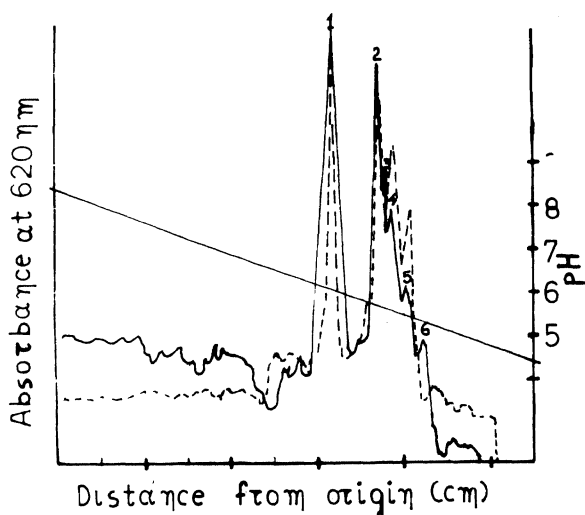


FIG. 1. Densitogram and pH gradient of slime glycolipoprotein (GLP) extracted from the gentamicin-sensitive parent strain of *P. aeruginosa* C2 (---), and its laboratory-induced resistant variant (—).

Our results suggest that the differences are also qualitative, at least in the protein component of the slime GLP. The two protein bands found in the slime GLP of the resistant variant might possibly be outer membrane proteins extracted with the GLP, though this possibility seems highly unlikely. The presence of two additional protein bands in the slime GLP of the resistant variant may have implications in its biological properties, such as its antigenicity. Alterations in the chemical composition and the biological properties of the slime GLP have been also reported following lysogenization of *P. aeruginosa* (Dimitracopoulos and Bartell 1979). Experiments are now in progress to demonstrate whether the laboratory resistance to gentamicin effects any other quantitative and (or) qualitative changes in the other components of slime GLP and its biological properties.

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