Inhibition of Swarming of *Proteus* by Sodium Tetrade cyl Sulfate, β-Phenethyl Alcohol, and *p*-Nitrophenylglycerol

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Received for publication 27 April 1966

**Abstract**

Kopp, Rudolf (Hygiene-Institut der Universität, Freiburg, Germany), Johannes Müller, and Renate Lemme. Inhibition of swarming of *Proteus* by sodium tetrade cyl sulfate, β-phenethyl alcohol, and *p*-nitrophenylglycerol. Applied Microbiol. 14:873–878. 1966.—The effects of sodium tetrade cyl sulfate (STS), β-phenethyl alcohol (PEA), and *p*-nitrophenylglycerol (PNPG) on motility, swarming, flagellation, and growth of *Proteus* were examined. Growth-inhibitory concentrations (GIC) and swarming-inhibitory concentrations (SIC) were determined. A characteristic of the swarming-inhibitory efficacy of these compounds was based on their GIC/SIC ratio and their concentration inhibition curves. Using the homologous series of sodium alkyl sulfates as a standard reference, we showed that PNPG was more effective than STS, which was the most effective of the homologous series. PEA was less effective than sodium decyl sulfate but more effective than sodium octyl sulfate. Motility tests in liquid medium and electron microscope investigations indicated that the modes of action of the three compounds, all of which effectively inhibit the swarming of *Proteus*, are different. Whereas STS and PEA inhibit swarming by inhibition of motility, PNPG seems to act on the swarming mechanism sensu strictior, without impairment of motility. STS immobilizes by inhibition of flagellum formation or by some lytic action on the flagella already synthesized. PEA acts by impairing flagellar function, but leaves the flagella morphologically intact.

A number of compounds have been proposed as additives to solid media to prevent the swarming of *Proteus*. The results of investigations concerning the effects of three of these compounds, sodium tetrade cyl sulfate (STS), β-phenethyl alcohol (PEA), and *p*-nitrophenylglycerol (PNPG), on motility, swarming, flagellation, and growth of *Proteus* are presented in this paper.

STS (C14) was previously reported to be the most effective antiswarming agent of the homologous series of sodium alkyl sulfates with an even number of carbon atoms (6). PEA was used as an antiswarming agent by Jawetz (personal communication). According to Berrah and Konetzka (2), PEA inhibits deoxyribonucleic acid (DNA) synthesis. In a later study (11), Treick and Konetzka found that PEA in a concentration of approximately 25 mM inhibits only the initiation of DNA replication without affecting the polymerization events once the process has started. They suggested that PEA causes a physical alteration of the bacterial membrane which affects the attachment of the chromosome to the membrane. Rosenkranz et al. (9, 10) reported that messenger ribonucleic acid (RNA) is selectively inhibited when low levels (0.05%) of PEA are used. At concentrations of PEA above 0.2%, the synthesis of all major biopolymers appears to be arrested. According to White and White (12), PEA also seems to interfere with electron-transport mechanisms. PNPG was used by Beer (1) to prevent the swarming of *Proteus*. Kopp and Lemme later reported on the inhibition of swarming by PNPG and stereoisomers of the chloramphenicol base (5).

**Materials and Methods**

*P. mirabilis* strain 21, isolated from human urine and kept on agar slants stored at 4°C, was transferred to a liquid medium and then streaked on a solid medium before it was used in the experiments. Three compounds were employed in the experiments: (i) STS, an anionic detergent obtained by courtesy of Prof. Blaser, Henkel AG, Düsseldorf, Germany, (ii) PEA, obtained from Fluka AG, Buchs SG, Switzer-
land, and (iii) PNPG, obtained from Fa. Carl Roth, Karlsruhe, Germany.

**Solid medium.** In the experiments on solid medium, the effect of the three compounds on the swarming phenomenon was examined as described previously (6). The medium employed in the previous study was used with the pH adjusted to 7.2. The swarming distance (maximal distance between the edge of the parent colony and the peripheral ring of growth) was read after 12 hr in some experiments, and at 2-hr intervals in others. In addition, the concentrations of the compounds completely inhibiting growth were determined. For this purpose, the plates were read after 12 hr. STS was added to the medium to final concentrations of 0.1 to 1.0 mM, PEA in the concentration range of 5 to 50 mM, and PNPG in the range of 0.05 to 0.2 mM (to prevent swarming) and 1 to 3 mM (to prevent growth). Electron microscopy was conducted as previously described. The three compounds were added to the medium in final concentrations of 0.5 mM for STS, 10 mM for PEA, and 0.2 mM for PNPG.

**Liquid medium.** For these experiments, a complex medium containing 0.5% glucose, 0.5% Casamino Acids (Difco), 0.5% NaCl, 0.025% MgSO₄·7 H₂O, and potassium-phosphate buffer (pH 7.2) was employed. The organisms were grown in test tubes (18 mm in diameter) containing 10 ml of the medium. The tubes were inoculated with 0.5 ml of a 12-hr culture and incubated at 37 C. STS was added to the medium to final concentrations of 0.1, 0.2, 0.5, and 1 mM; PEA was added in the concentration range of 10 to 50 mM and PNPG, from 0.5 to 10 mM. Growth was followed nephelometrically with a Zeiss photometer (Elko III). Uninoculated tubes served as sterility controls and were used to zero the photometer. The nephelometric measurements were standardized by viable counts. Motility was studied with the hanging-drop technique. Samples were taken at 2-hr intervals. The control (not containing inhibitors) was taken as 100% motile. The motility in the control was optimal after 4 to 6 hr and remained so for the rest of the experiment. For the electron microscope investigations in liquid medium, the inhibitors were added to the medium in the following concentrations: STS, 0.1 mM; PEA, 20 and 50 mM; PNPG, 100 mM. The inhibitors were added in solution to 50 ml of logarithmically growing cultures. Samples (5 ml) were taken from the culture 1 min after addition of the compounds and 6 hr later. The samples were centrifuged and washed with saline. For electron microscopy the sediment was suspended in 0.1% phosphotungstic acid and sprayed on a grid covered with Formvar film. The control observations on motility were performed by the hanging-drop technique.

**RESULTS**

**Solid medium.** Growth-inhibitory concentrations (GIC), swarming-inhibitory concentrations (SIC), and GIC/SIC ratios of STS, PEA, and PNPG are given in Table 1. The concentration of the inhibitors which completely prevented swarming of Proteus over a 12-hr period with only a slight impairment of growth was 0.5 mM for STS and 0.2 mM for PNPG. For PEA, this concentration was considerably higher (10 mM), and at this concentration growth was also considerably impaired. Growth was completely inhibited by 30 mM PNPG and 50 mM PEA. The GIC of STS could not be determined exactly. At saturation point (about 1 mM at 37 C), STS impaired growth considerably, but there was no complete inhibition. The GIC of STS is certainly higher than 1 mM and is probably lower than 5 mM.

Figure 1 shows the inhibitory effects of various concentrations of STS, PEA, and PNPG. The swarming distance is given as a function of time. Figure 2 is partly adapted from a previous report (6). These concentration response curves can be used to compare the inhibition of swarming by different compounds. By using the homologous series of the sodium alkyl sulfates as a standard reference, we have shown that PNPG is slightly more effective than STS, which is the most effective of the sodium alkyl sulfates, and that the effectiveness of PEA lies between sodium octyl sulfate and sodium decyl sulfate. The results of the electron microscope investigations on solid medium are given in Table 2. At a concentration of STS which completely inhibited the swarming phenomenon, no flagella could be detected. At concentrations of PEA and PNPG which completely inhibited swarming, flagella were morphologically intact.

**Liquid medium.** The results of the experiments in liquid medium are presented in Fig. 3 and Table 3. Growth and motility of the bacteria were checked over a 12-hr period at various concentrations of the inhibitors.

It is obvious from Fig. 3a that a concentration of STS which impairs or prevents the swarming of Proteus on solid medium also inhibits motility in liquid medium. To achieve immobilization in liquid medium, higher concentrations of PEA were required than on solid medium. A concentration of 10 mM PEA, which completely pre-

**Table 1. Effect of STS, PEA, and PNPG on growth and swarming of Proteus on solid medium**

<table>
<thead>
<tr>
<th>Compound</th>
<th>GIC&lt;sup&gt;a&lt;/sup&gt; (mM)</th>
<th>SIC&lt;sup&gt;a&lt;/sup&gt; (mM)</th>
<th>GIC/SIC&lt;sup&gt;b&lt;/sup&gt; ratio&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>STS</td>
<td>5</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>PEA</td>
<td>50</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PNPG</td>
<td>30</td>
<td>0.2</td>
<td>150</td>
</tr>
</tbody>
</table>

<sup>a</sup> GIC = growth-inhibitory concentration; SIC = swarming-inhibitory concentration.  
<sup>b</sup> Equals the efficacy index.
INHIBITION OF SWARMING OF PROTEUS

Fig. 1. Inhibition of swarming of Proteus by various concentrations of sodium tetradecyl sulfate (a) β-phenethyl alcohol (b), and p-nitrophenylglycerine (c).

Table 2. Effects of STS, PEA, and PNPG on swarming and flagellation of Proteus on solid medium

<table>
<thead>
<tr>
<th>Compound</th>
<th>Swarming impaired at m M</th>
<th>Swarming completely inhibited at m M</th>
<th>Flagellation at inhibitory concn</th>
</tr>
</thead>
<tbody>
<tr>
<td>STS</td>
<td>0.1</td>
<td>0.5</td>
<td>No flagella</td>
</tr>
<tr>
<td>PEA</td>
<td>5</td>
<td>10</td>
<td>Flagella</td>
</tr>
<tr>
<td>PNPG</td>
<td>0.05</td>
<td>0.2</td>
<td>Flagella</td>
</tr>
</tbody>
</table>

...vented the swarming phenomenon over a 12-hr period, had only a very short-lived effect on motility in liquid medium. At 1 hr after inoculation, all cells were immobilized, whereas after 2 hr more than 50% of the cells were motile. At a concentration of 30 mM, complete immobilization was achieved up to 4 hr after inoculation. Between 6 and 12 hr, more than 50% of the cells were immotile, but complete immobilization was not achieved.

STS and PEA immobilized Proteus cells both on solid medium and in liquid medium, but PNPG had no immobilizing effect whatsoever in liquid medium, even at a concentration (5 mM) 25 times higher than the SIC. Even at 10 mM, no immobilizing effect was observed during the first 6 hr of growth (Fig. 3c), although this concentration was almost bacteriostatic. Only after 8 to 12 hr could a certain immobilizing effect be recognized, but complete inhibition of motility was not attained.

Increasing concentrations of PEA impaired both motility and growth of Proteus (Fig. 3b). In
contrast, STS at a relatively low concentration (0.1 mM) inhibited the motility of *Proteus* without impairing growth. Only at a higher concentration (0.5 mM) was growth impaired, and at a still higher concentration (1 mM) growth was inhibited.

The results of the electron microscope investigations in liquid medium were as follows. Immediately after addition of STS to the culture to a final concentration of 0.1 mM, flagellation was normal and the motility of the cells was not impaired. After 6 hr, the cells had lost their flagella, and motility was completely inhibited. After 12 hr, the cells were still immobilized. After the addition of PEA to a final concentration of 20 and 50 mM, on the other hand, the cells were immobilized immediately, but flagella remained morphologically intact. After 6 hr, flagella were still intact morphologically at both concentrations. At a concentration of 20 mM, the cells were motile again after 6 hr, whereas at a concentration of 50 mM they remained immotile over the full period of 12 hr. When PNPG was added to the culture at a concentration of 100 mM, which is 500 times higher than the concentration necessary to prevent swarming, the cells were fully motile immediately after addition of the inhibitor, and flagella were intact morphologically. After 6 hr, almost all cells were immobilized, and flagellation was impaired, but some flagellated cells could still be observed.

**DISCUSSION**

The present paper deals with two different aspects of inhibition of swarming of *Proteus*: first, the proper evaluation of the efficacy of different compounds used to prevent the swarming phenomenon, and, second, different modes of actions of these agents.
The concentration inhibition curves of the homologous series of sodium alkyl sulfates (6) should be used as the references for swarming-inhibitory efficacy of different compounds (Fig. 2). The concentration inhibition curve, however, although useful for comparing different compounds, is not sufficient to satisfactorily characterize these compounds as swarming-inhibitory agents. The ratio of GIC to SIC, which could be called the efficacy index EI, also must be taken into account (Table 1). A high EI indicates that only a low concentration of a compound relative to the concentration necessary to produce inhibition of growth is required to inhibit swarming. A low EI, on the other hand, indicates that SIC and GIC lie relatively close together. The very high EI of PNPG suggests a rather specific mode of action of the compound as an inhibitor of the swarming phenomenon of Proteus, whereas the low EI of STS and PEA suggest a nonspecific effect. Another clue that different modes of action are involved is the observation that flagellation is unimpaired when PEA or PNPG is used at SIC, whereas no flagella can be detected when STS is employed to prevent the swarming phenomenon. It had been previously concluded (6) that detergents such as STS prevent the swarming of Proteus either by inhibition of flagellum formation or by some lytic action on the flagella already synthesized.

PNPG, which leaves flagella intact morphologically and does not impair flagellar function, seems to interfere with the mechanism of swarming in the strict sense. According to Lominski and Lendrum (8) and Hughes (3), the swarming phenomenon is based on negative chemotaxis caused by metabolites of certain bacteria. If the concentration of the hypothetical metabolites reaches a certain level within the colony of motile bacteria, the swarming phenomenon is elicited. The bacteria move along the concentration gradient of the metabolites away from the mother colony. Similar explanations for the swarming phenomenon have been offered by Klieneberger-Nobel (4) and Kvittingen (7). If these explanations are correct, it is conceivable that PNPG acts by inhibiting the formation of metabolites which elicit negative chemotaxis, or by modifying the response of the bacteria to the metabolites.

Although STS destroys flagella and PNPG possibly interferes with the mechanism of negative chemotaxis underlying the swarming phenomenon, it is feasible that PEA acts by some alteration of the bacterial membrane which might bear on flagellar function. Treick and Konetzka (11) have reported that PEA in a concentration of approximately 25 mM inhibits the initiation of DNA replication, and they have suggested that this inhibition is caused by a physical alteration of the bacterial membrane which affects the attachment of the chromosome to the membrane. Our own data indicate that a substantial impairment of motility in liquid medium (Fig. 3) by 20 to 40 mM PEA is paralleled by an inhibition of growth. The source of energy and the energy-transport mechanisms involved in flagellar function are as yet poorly understood. However, it is well established that flagella originate in the cytoplasm and pass through the bacterial membrane, and it is conceivable that a certain intact state of this membrane is a prerequisite for normal flagellar function. It is possible that the physical alteration of the bacterial membrane by PEA, which inhibits the initiation of DNA replication, at the same time might impair flagellar function.

It is concluded that the swarming phenomenon of Proteus can be inhibited (i) by interference with flagellum formation or destruction of flagella already synthesized (e.g., sodium alkyl sulfates), (ii) by interference with flagellar function, possibly via some physical alteration of the bacterial membrane (PEA), and (iii) by interference with the mechanism of swarming sensu strictiori, possibly via some interference with a mechanism of negative chemotaxis proposed by Lominski and Lendrum (8).

**LITERATURE CITED**


