Studies on the Pyocins of *Pseudomonas aeruginosa*: Morphology and Mode of Action of Contractile Pyocins

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SUMMARY

The adsorption and inhibitory action of pyocin 21 on sensitive cells of *Pseudomonas aeruginosa* (*P. pyocyanea*) were observed by fluorescence microscopy and by using agar cultures. By electron microscopy, preparations from four pyocinogenic strains of *P. aeruginosa* were seen to include contracted and uncontracted particles, isolated tail cores and sheaths. The importance of adsorption and contraction of the particles on the surface of sensitive bacteria for the inhibitory action of these pyocins is suggested by the following evidence: (1) Particles adsorbed to sensitive bacteria at 0 °C, but contraction of particles was not then observed and the bacteria were not disrupted. (2) Pyocin particles did not adsorb to pyocin-resistant bacteria. (3) The ability to adsorb to sensitive bacteria and to kill them was absent after removal of the contractile sheath with sodium dodecyl sulphate. (4) When treated with homologous antiserum, pyocin activity was neutralized and the agglutinated particles remained unadsorbed and uncontracted. (5) Bacterial receptors for contractile pyocins contain lipopolysaccharide; such extracts from sensitive, but not from resistant, cells of *P. aeruginosa* inactivated pyocins, and contracted particles were seen adsorbed to lipopolysaccharide fragments.

INTRODUCTION

Bacteriocins are antibiotic substances produced by certain strains of bacteria and differ from classical antibiotics in that their lethal activity is restricted to other strains of the same or closely related species. Jacob (1954) described a bacteriocin from a strain of *Pseudomonas aeruginosa* and called the substance pyocin; synthesis of the pyocin, C10, was inducible with u.v. irradiation. Kageyama (1964) reported that mitomycin C could induce the synthesis of pyocin R and by electron microscopy he demonstrated rod-like particles in a purified pyocin preparation. Further electron micrographs of pyocins (Ishii, Nishi & Egami, 1965; Higerd, Baechler & Berk, 1967; Govan, 1968) have revealed that one type of pyocin resembles headless contractile bacteriophages, e.g. the T-even coliphages, consisting of a contractile sheath surrounding an inner core. Higerd, Baechler & Berk (1969) demonstrated attachment of pyocin C9 to bacterial wall fragments and reported an apparent correlation between uncontracted particles and lethal activity. Yui, Ishii & Egami (1969) reported that the lethality of pyocin R was lost after treatment with p-chloromercuribenzoate and restored by addition of ¿-mercaptoethanol; electron microscopy revealed a corresponding change in the proportion of contracted to uncontracted particles.

The importance of *Pseudomonas aeruginosa* as an opportunist pathogen has become increasingly obvious in recent years and the incidence of Pseudomonas infections has also risen considerably (Asay & Koch, 1960). Holloway (1960) reported that pyocinogeny occurred frequently in strains of *P. aeruginosa* and suggested that pyocin production might
be a suitable epidemiological marker for this species. Such a method of characterizing strains of *P. aeruginosa*, pyocin typing, employs a standard set of strains of *P. aeruginosa* to detect pyocinogeny in a test strain (Gillies & Govan, 1966; Govan & Gillies, 1969); the method is now used in many centres (Phillips, Lobo, Fernandes & Gundara, 1968; Csiszar & Lanyi, 1970; Ziv, Mushin & Tagg, 1971; Neussel, 1971).

This paper describes the morphology and mode of action of several contractile pyocins obtained from strains of *Pseudomonas aeruginosa* belonging to different pyocin types.

**METHODS**

The pyocinogenic strains used were *Pseudomonas aeruginosa* 21, 355, 149 and 430; these belong to pyocin types 1, 3, 9 and 10 respectively (Gillies & Govan, 1966). The eight indicator strains of *P. aeruginosa* (1 to 8) used in the pyocin-typing technique (Gillies & Govan, 1966) were used to assay pyocin activity.

**Extraction of non-induced pyocin by freezing and thawing.** The pyocinogenic strain of *Pseudomonas aeruginosa* was inoculated over the entire surfaces of several Petri dishes containing tryptone soya agar (Oxoid) and the plates were incubated for 18 h at 32°C; thereafter they were frozen at −70°C for 1 h and then allowed to thaw at room temperature before the expressed fluid was removed with a sterile Pasteur pipette and centrifuged at 1200 g. The supernatant, containing pyocin activity, was treated with 5% (v/v) chloroform to kill any remaining bacteria.

**Induction of pyocin in fluid media.** The medium used was tryptone soya broth (Oxoid) or sodium glutamate broth (Kageyama & Egami, 1962). A 2 ml portion of a static overnight culture of the pyocinogenic strain was added to 200 ml of sterile broth and incubated with agitation for 2 to 3 h at 32°C. Mitomycin C was added to a final concentration of 1 μg/ml and incubation continued until lysis of the culture occurred, normally after a 3 to 4 h incubation period. The lysate was centrifuged at 2400 g for 30 min to remove bacterial debris and the supernate treated with 5% (v/v) chloroform. The supernatant fraction was designated crude pyocin. Control cultures containing no mitomycin C were also investigated.

**Purification of pyocins.** Crude pyocin lysates were purified by a modification of the method of Kageyama & Egami (1962).

After treatment with 5% (v/v) chloroform, one litre of lysate was treated with 60 ml of m-MnCl₂·4H₂O added slowly during agitation and the pH adjusted to 7.5 with N-sodium hydroxide. The precipitate containing viscous material was removed by centrifugation at 2400 g for 15 min. The supernatant fraction was designated 'partially purified pyocin'. Further purification was carried out by adding ammonium sulphate to 70% saturation and allowing it to stand overnight at 4°C. The precipitate, containing pyocin activity, was collected by centrifugation for 30 min at 2400 g at 4°C. The precipitate was dissolved in 50 ml of 0.01 M-tris (hydroxymethyl) methylamine buffer (pH 7.5) containing 0.01 M-MgCl₂·6H₂O and 0.01 M-MgSO₄·7H₂O, and dialysed against 21 of the same buffer overnight at 4°C. If necessary the resulting preparation was clarified by centrifugation (2400 g for 15 min at 4°C).

The pyocin preparation was then centrifuged for 90 min at 100000 g under refrigeration (Spinco, type 40 rotor). The gelatinous sediment was gently dissolved in 20 ml of buffer and chromatographed on DEAE-cellulose (Pharmacia, Uppsala, Sweden) previously washed and equilibrated with the same buffer. An 8 ml sample of pyocin was applied to the column (28 × 1.5 cm) and allowed to adsorb for 1 h; 200 ml of buffer were passed
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through the column to remove material not adhering to the cellulose and the sample was then eluted with 800 ml of a sodium chloride gradient (0 to 1.0 M in 0.01 M-buffer). The u.v. absorbancy of fractions at 280 nm was measured with a Unicam SP 500 spectrophotometer and, after assay for pyocin activity, appropriate fractions were dialysed against tris buffer to remove sodium chloride. This purified pyocin was then concentrated by a further cycle of ultracentrifugation.

**Assay of pyocin activity.** A 4 h nutrient broth culture at 37 °C of a pyocin-sensitive strain of *Pseudomonas aeruginosa* (containing approximately 10⁸ organisms/ml) was used to flood the surface of a nutrient agar plate (9 ml of medium contained in an 8.5 cm plastic Petri dish), excess broth culture was removed with a sterile Pasteur pipette and the bacterial lawn allowed to dry at room temperature. Doubling dilutions of the pyocin preparation were made in sterile saline (0.85 ‰) and a drop of each dilution applied to the surface of the plate by means of a calibrated platinum-tipped pipette delivering 0.02 ml per drop. When the drops had dried the plates were incubated overnight at 37 °C. The titre of pyocin activity (units/ml) was read as 50 times the reciprocal of the highest dilution causing complete inhibition of the sensitive strain.

**Agar-slide technique.** Sterile nutrient agar was poured into a channel, measuring 8.0 × 2.2 × 0.1 cm, in a mould. Before the agar gelled, glass microscope slides were placed at right-angles across the agar channel, taking care that no air bubbles formed. After 15 min when the agar had gelled, the slides were inverted bearing a uniform block of nutrient agar, and excess agar was cut away.

A drop of fluid taken from a 4 h nutrient broth culture of *Pseudomonas aeruginosa* strain 6, 8 or 21 was placed on the agar block together with a drop of pyocin 21 preparation (3200 units/ml) obtained by the freezing and thawing technique. After absorption, a cover glass was applied to the agar surface, excess agar removed with a scalpel and the edges of the block sealed with molten paraffin wax. The preparation was incubated at 37 °C in a moist chamber and examined at regular intervals by phase-contrast microscopy in a Leitz Ortholux microscope, Vernier readings being used to locate fields of view.

**Preparation of pyocin antisera.** Rabbits were inoculated subcutaneously with 1 ml volumes, containing 0.5 ml of purified pyocin 21, titre 200 000 units/ml, mixed with an equal volume of Freund's complete adjuvant; another rabbit received the same amount of pyocin subcutaneously, without adjuvant, and a similar dose after seven days. Rabbits were bled before immunization and at various intervals afterwards.

**Effect of antiserum on pyocin activity.** Doubling dilutions of antiserum were prepared in 0.25 ml volumes of sterile saline. To each dilution was added 0.25 ml of pyocin 21, twice as much as caused confluent lysis of the sensitive strain 8. The mixtures were incubated at 37 °C in a water bath for 1 h and then assayed for pyocin activity.

**Effect of lipopolysaccharide on pyocin activity.** Ether-extracted lipopolysaccharide (LPS) from the pyocin-sensitive strains 1 and 8 was dissolved to a concentration of 4 mg/ml in 1 M-ammonium acetate. Then 100 000 units of partially purified pyocin (21 or 430) in 0.5 ml of 0.1 M-ammonium acetate were mixed with 0.5 ml of LPS and incubated in a water bath at 37 °C for 1 h. Doubling dilutions were made in sterile saline and the titre of pyocin activity measured. Pyocin 21 was also examined after treatment with LPS from the resistant strain 6.

**Effect of sodium dodecyl sulphate on pyocin activity.** Doubling dilutions of 2% sodium dodecyl sulphate (SDS) were prepared in 0.5 ml volumes of distilled water and 100 000 units of partially purified pyocin 21 in 0.5 ml volumes were added to each dilution. The mixtures were incubated at 37 °C in a water bath for 1 h and assayed for pyocin activity.
Fluorescent antibody studies. A 0.5 ml volume of purified pyocin 21 containing 100000 units/ml was added to 2 ml of a 4 h peptone–water culture of strains 6, 8 or 21. After 10 min at 37 °C the preparation was centrifuged for 45 min at 2850 g and the bacterial cells resuspended in 2 ml of 0.01 M-tris buffer. Then 0.5 ml of the gamma globulin component of pyocin antiserum (neutralization titre of 4000), purified according to the method of Campbell, Garvey, Cremer & Sussdorf (1963), was added; the contents were incubated for a further 45 min at 37 °C, recentrifuged, and the pellet suspended in 1 ml of 0.01 M-phosphate buffer (pH 7.1). A heat-fixed smear was prepared on a glass slide and covered with anti-rabbit gamma globulin labelled with fluorescein isothiocyanate (FITC, Hoechst, Marburg) for 30 min. The smear was washed twice (5 min) in phosphate buffer and finally in distilled water. A Leitz Ortholux microscope, fitted with an HBO 200 lamp and a UG I filter, was used.

Electron microscopy. A negative-staining technique (Brenner & Horne, 1959) was used. Pyocin preparations were centrifuged for 90 min at 100000 g under refrigeration (Spinco, type 40 rotor). The sediment was resuspended in 1 M-ammonium acetate, pH 7.0. Equal volumes of this suspension and 2 % potassium phosphotungstate at pH 7.0 were mixed on the surface of a clean glass slide and a drop of the mixture transferred to the surface of a grid. After 30 s, excess fluid was removed and the preparation allowed to dry.

In order to observe the effects of contractile pyocin on sensitive and resistant strains of Pseudomonas aeruginosa, 0.5 ml of induced partially purified pyocin 21 (400000 units/ml) was added to 3.5 ml of a 4 h peptone–water culture of strains 8 or 6. After incubation at 37 or 0 °C for various durations the suspension was fixed in 4 % formaldehyde, centrifuged at 2850 g for 45 min and resuspended in 1 M-ammonium acetate. When the resistant strain 6 was treated with pyocin, centrifugation was also carried out at 100000 g for 90 min to precipitate both cells and pyocin particles.

To observe the interaction of pyocin and antisera, 0.5 ml of pyocin 21 antiserum (neutralization titre 1:500) and 0.5 ml of partially purified pyocin 21 (50000 units/ml) were mixed and incubated in a water bath for 1 h at 37 °C. To this was added a 3.5 ml vol. of a 4 h peptone–water culture of the sensitive strain 8 (10⁸ organisms/ml) and the mixture held for a further 15 min at 37 °C before fixation with 4 % formaldehyde. After centrifugation for 90 min at 100000 g the sediment was resuspended in 1 M-ammonium acetate.

To observe the interaction of pyocins and LPS, 0.5 ml samples of pyocin and LPS (described earlier) were mixed and held for 1 h at 37 °C. Each suspension was centrifuged at 41000 g for 45 min and the sediment resuspended in ammonium acetate.

Electron micrographs were made at 50 kV using Ilford Special Contrasty plates.

RESULTS

Non-induced production of pyocin. Non-induced pyocins, extracted as described earlier from agar cultures of Pseudomonas aeruginosa strains 21, 355, 149 and 430, were tested for inhibitory activity against the eight indicator strains of P. aeruginosa used in the standard pyocin-typing technique. The patterns of inhibition which resulted were identical to those obtained with the same pyocinogenic strains using the standard pyocin-typing method. Similar results were obtained when the pyocinogenic strains were grown for 18 h in tryptone soya broth or sodium glutamate broth and the cell-free extracts tested for pyocin activity. When assayed quantitatively the production of pyocin by the four strains in agar or broth cultures reached a maximum of approximately 3200 units/ml after 12 h growth at 32 °C.

Induction of pyocin with mitomycin C. The addition of mitomycin C to give a final concen-
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Fig. 1. Pyocin induced from Pseudomonas aeruginosa 355. Uncontracted, bullet-like particles possessing a base-plate (a), and contracted particles (b) consisting of a hollow or partially-filled core surrounded by a sheath can be seen. Short fibres extend from the base of the contracted sheath and the other end is sealed or partially open. Ringlets resembling minute cogwheels are also visible (c). Bar marker = 200 nm.

A concentration of 1 μg/ml in cultures of the pyocinogenic strains in the logarithmic phase led to a considerable increase in pyocin production. After bacterial lysis, crude pyocin preparations were regularly obtained with a titre of 400 000 units/ml. After purification and ultracentrifugation, pyocin titres of $2.5 \times 10^7$ units/ml could be obtained. The high-strength pyocin preparations obtained from these four pyocinogenic strains after induction with mitomycin C gave the same inhibition patterns against the eight indicator strains as did pyocin preparations obtained from non-induced cultures of the organisms.

Serological response to pyocin. After a single subcutaneous injection of purified pyocin 21 in Freund’s complete adjuvant, the neutralization titre of sera from rabbits against the homologous pyocin rose from 1000 after nine days to a maximum of 32 000 after two months. When a subcutaneous inoculation of pyocin was given without adjuvant and a second administered seven days later, a maximum titre of neutralizing antibodies of 1000 was reached 14 days after the second injection.

Pyocin and agar slide cultures of Pseudomonas aeruginosa. The inhibitory activity of non-induced pyocin 21, obtained by the freezing and thawing technique, was examined on agar slide cultures of P. aeruginosa indicator strains 6 and 8 which are respectively resistant and sensitive to this pyocin. Cells of indicator strain 8 failed to multiply in the presence of pyocin 21. In contrast, no activity was noted against cells belonging to indicator strain 6 or to the pyocinogenic strain 21.
Fig. 2. Pyocin induced from *Pseudomonas aeruginosa* 21. A helical substructure is suggested in the uncontracted particle (a). The contracted particle (b) shows a hollow tail core enclosed in a sheath closed at one end and possessing hook-like fibres at the other. A discarded empty sheath is also visible (c). Bar marker = 200 nm.

Fig. 3. Contracted pyocin particle in induced preparation from *Pseudomonas aeruginosa* 149; the partial disengagement of a tail core from its sheath is visible. Bar marker = 200 nm.

**Detection of pyocin adsorption to sensitive bacteria by using a fluorescent-antibody technique.** Cells of indicator strain 8 after reaction with pyocin 21, fluoresced when treated with a gamma globulin component of homologous antiserum followed by anti-rabbit gamma globulin conjugated with FITC. No fluorescence of bacteria was observed if indicator strain 6, or the pyocinogenic strain 21, was used, (ii) in the absence of pyocin, or (iii) when pre-immune gamma globulin was used.

**Electron microscopy of pyocin preparations**

Electron microscopy of induced, partially purified pyocin preparations from the four pyocinogenic strains of *Pseudomonas aeruginosa* revealed the presence of numerous structures resembling the tail components of contractile bacteriophages. Both uncontracted and contracted forms were visible (Fig. 1). Contracted particles consisted of a core partially surrounded by a sheath. In most particles the core appeared empty but occasionally cores were seen to contain material. Uncontracted particles resembled bullets and a base-plate was visible at the broader end. Hook-like pins extended from the lower ends of contracted sheaths and occasionally several fibres were seen attached to the base-plate. Isolated
Fig. 4. *Pseudomonas aeruginosa* indicator strain 8 after 1 min contact (37 °C) with induced pyocin 21. The bacterial surface is surrounded by uncontracted pyocin particles. Bar marker = 200 nm.
Fig. 5. *Pseudomonas aeruginosa* indicator strain 8 after 20 min contact at 37 °C with induced pyocin 21. The bacterial surface appears convoluted and covered with many pyocin particles. Contracted particles are visible (a) attached to the bacterial surface, and several uncontracted particles (b) can be seen close to the bacterial surface. Many discarded cores (c) and empty sheaths (d) are present in the vicinity of the bacterium. Bar marker = 200 nm.
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Contracted sheaths were observed and long sheath-like structures were seen composed of numerous connected sheaths. Hollow ringlets resembling minute cogwheels were also observed. The extended particles possessed a helical or horizontally striated substructure of approximately 20 subunits (Fig. 2). The partial disengagement of a tailpiece from its collar sheath is shown in Fig. 3. The majority of particles, regardless of the pyocinogenic strain used, measured approximately $100 \times 15$ nm in the uncontracted state; contracted particles consisted of a hollow tailpiece, approximately $100 \times 7$ nm, partially enclosed in a sheath measuring about $45 \times 17$ nm.

When pyocin extracts were prepared from non-induced cultures of the pyocinogenic strains, similar though fewer particles were observed. Intact bacteriophage particles were not observed in preparations from any of the strains examined.

**Relationship between lethal activity and the nature of pyocin particles**

The adsorption and inhibitory activity of pyocin 21 towards sensitive strains of *P. aeruginosa* was investigated by fluorescence microscopy and the agar-slide technique; by using the electron microscope, additional features of this activity could be seen: (1) Uncontracted particles attached rapidly by the broader or base-plate end to sensitive bacteria. (2) If fixation was carried out immediately after addition of pyocin or if the system was held at $0^\circ C$, no contraction of adsorbed particles was observed (Fig. 4). Fig. 5 shows a cell of *P. aeruginosa* indicator strain 8, 20 min after the addition of pyocin; the entire surface of the bacterium appears smothered with rod-like particles. A number of empty sheaths and detached tailpieces can be detected in the vicinity of the bacterium. The surface of the bacterium is grossly irregular as if disruption were imminent and, indeed, after 2 h incubation with the pyocin preparation, more than $90\%$ of the bacteria were seen to be completely disrupted.

**The importance of contraction in pyocin activity**

The pyocin produced by *Pseudomonas aeruginosa* strain 21 had no inhibitory effect on indicator strain 6 when tested by the standard pyocin-typing method or at higher concentrations after induction and purification. When pyocin 21 was mixed with indicator strain 6 (Fig. 6) and the system examined in the electron microscope, no attachment of particles to the surface of bacteria was observed and most of the particles remained uncontracted. The inhibitory activity of pyocin 21 against indicator strain 8 was destroyed after treatment with $0.02\%$ SDS. Electron microscopy showed that the particles had lost the outer contractile sheath and did not adsorb to the surfaces of sensitive bacteria. No change in lethal activity of pyocin or morphological alteration was observed after treatment with SDS at concentrations less than $0.01\%$.

Homologous antiserum neutralized the activity of pyocin 21, and in the electron microscope (Fig. 7) agglutinated particles appeared in the uncontracted state and no particles were found adsorbed to sensitive bacteria.

**The role of bacterial LPS as a receptor for contractile pyocins**

After adding contractile pyocins 21 or 430 to sensitive cells of *Pseudomonas aeruginosa*, rosette-like formations of contracted particles were often observed attached either to the bacteria (Fig. 8) or to cell debris (Fig. 9). When a pyocin preparation was mixed with LPS from the sensitive strains of *P. aeruginosa* the pyocin was inactivated and electron microscopy (Fig. 10) revealed the attachment of contracted particles to LPS fragments. No loss of
Fig. 6. Pseudomonas aeruginosa indicator strain 6 in the presence of induced pyocin 21. Indicator strain 6 is resistant to pyocin 21; the pyocin particles did not adsorb to the bacterial surface and most particles remained uncontracted. Bar marker = 200 nm.
Fig. 7. Pseudomonas aeruginosa indicator strain 8 in the presence of induced pyocin 21 which had previously been treated with specific antiserum. Indicator strain 8 is sensitive to pyocin 21; however, no attachment of pyocin to the bacterial surface occurred and the agglutinated particles remained uncontracted. Bar marker = 200 nm.
Fig. 8. *Pseudomonas aeruginosa* indicator strain 8 after treatment with induced contractile pyocin 430. Several rosettes of contracted pyocin particles are formed around rounded fragments at the bacterial surface. Bar marker = 200 nm.

Fig. 9. Rosettes of contracted, induced pyocin 430 surrounding cell material from *Pseudomonas aeruginosa* indicator strain 8. Bar marker = 200 nm.

Fig. 10. Contracted particles of induced pyocin 430 adsorbed to lipopolysaccharide from *Pseudomonas aeruginosa* indicator strain 1. Bar marker = 200 nm.

Pyocin 21 activity occurred nor was attachment of the particles observed when this pyocin was mixed with LPS from the resistant strain.

**DISCUSSION**

The adsorption of pyocin to sensitive cells of *Pseudomonas aeruginosa* can be demonstrated in the light microscope by using a fluorescent antibody technique and the inhibitory
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activity demonstrated by the agar-slide technique. Electron microscopy, however, reveals in greater detail the nature of these pyocins and their possible mode of action. The contractile pyocins of *P. aeruginosa* strains 21, 355, 149 and 430 resemble morphologically pyocin R (Ishii *et al.* 1965), pyocin C9 (Higerd *et al.* 1967), pyocin C10 (Bradley, 1967) and bacteriocins from *Proteus* (Coetsee, de Klerk, Coetsee & Smit, 1968) and *Vibrio* (Jayawardene & Farkas-Himsley, 1969).

The electron micrographs reproduced here suggest the sequence of events in pyocin activity and the significance of the various particles already described in preparations of contractile pyocins. Uncontracted particles rapidly attach by the base-plate to receptors on the surface of sensitive cells of *Pseudomonas aeruginosa*. Once established, contact is firm since absorbed particles are sedimented at 2850 g whereas a much higher gravitational force is required to sediment unadsorbed particles. After adsorption, there is a rapid contraction of the particles followed by disruption of the bacterium.

The importance of adsorption and contraction for pyocin activity is emphasised by several experimental findings: (1) At 0 °C particles adsorb to sensitive bacteria but do not contract and lysis of the bacteria is not observed. (2) In the presence of resistant bacteria pyocin particles do not adsorb to the cell surface but can be observed in the uncontracted state in the vicinity of the bacterium. (3) Lethality and the ability to adsorb to sensitive bacteria are lost after removal of the contractile sheath by SDS. (4) When treated with homologous antiserum, pyocins no longer adsorb to sensitive bacteria and the agglutinated particles remain uncontracted.

The receptors on the bacterial surface to which contractile pyocins adsorb appear to be in the LPS fraction. Ikeda & Egami (1969) reported that LPS extracted from pyocin-sensitive but not resistant cells of *Pseudomonas aeruginosa* inactivated pyocin R. Similarly LPS extracts have been shown to inactivate and adsorb a contractile bacteriocin from *Proteus vulgaris* (Smit, Hugo & de Klerk, 1969). The inability of bacteria of indicator strain 1 to adsorb pyocin 21 after treatment with EDTA is consistent with the view that the pyocin receptor contains LPS (Stewart & Young, 1971). Electron microscopy indicates that inactivation of contractile pyocins from *P. aeruginosa* strains 21 and 430 by LPS extracted from indicator strain 1 is due to the adsorption of pyocin particles to the LPS fragments.

Although they resemble contractile bacteriophages in many properties, the contractile pyocins do not replicate in sensitive host bacteria. In morphological terms, their lack of any structure resembling a bacteriophage head indicates a concomitant lack of the main reservoir of nucleic acid. Jacob (1954) reported that pyocin C10 was resistant to ribonuclease and deoxyribonuclease. By electron microscopy, Bradley (1967) observed that some core particles of this contractile pyocin contained material, possibly nucleic acid, and similar observations were made with the pyocins studied in this investigation (Fig. 1). The nature and function of this material remains unknown.

The mode of action of contractile pyocins after adsorption to the bacterial surface requires further investigation. Kinetic studies on the antibacterial activity of pyocin R (Kageyama, Ikeda & Egami, 1964) and pyocin 21 (Young, 1970) suggested that cell death can result from adsorption of a single pyocin particle. Kaziro & Tanaka (1965a, b) reported that pyocin R inhibited macro-molecular synthesis by inactivation of bacterial ribosomes. The relationship between pyocin 21 and other contractile pyocins requires investigation and further studies may reveal more than one mechanism for the activity of contractile pyocins.
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