Relationship Between Rhabidosome and Pyocin in *Pseudomonas fluorescens*

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SUMMARY

Rhabidosomal rods released from strain 3R of *Pseudomonas fluorescens* appear to be polymerized sheaths of pyocin which had a structure like that of T-even phage tails. This conclusion was based on the following observations: (1) Morphologically the rods have the same structure and diameter as the contracted sheath of R-type pyocin. (2) They resemble the polysheath of T-even phages. (3) Antigenically they are similar to R-type pyocin. (4) They are usually induced together with R-type pyocin but not with 28-type pyocin.

INTRODUCTION

Since the discovery of rod-shaped particles in *Saprospira* (Lewin, 1963), this kind of structure has been reported in several species of bacteria (Bradley, 1967; Yamamoto, 1967; Reichenbach, 1967; Ueda & Takagi, 1968; Clark-Walker, 1969). Such rods found within or liberated from bacterial cells have been given the name 'rhabidosomes' (Lewin, 1963). Pate, Johnson & Ordal (1967) suggested that they were degenerate forms of the membranous structure of host cells. Ueda & Takagi (1968) explained rods in *Clostridia* in the same way. Recently many such structures have been reported in mitomycin-induced lysates of several species of bacteria (Bradley, 1967; Iida & Inoue, 1968; Clark-Walker, 1969); these rhabidosomes often appeared together with bacteriocins which had the structure of phage tails, and their origin might be different from that of the first type (Reichenbach, 1967; Clark-Walker, 1969). Many members of the *Pseudomonas* group of bacteria are known to be pyocinogenic (Hamon, 1956). During the course of experiments on pyocin we discovered that some of them also produced the second type of rhabidosome. The relation between pyocin and rhabidosome was investigated and is reported in this paper.

METHODS

Bacteria. Strain 3R of *Pseudomonas fluorescens* isolated in our laboratory was used as the rhabidosome-producing organism. Activity of the released pyocin was titrated on the C14 strain of *P. aeruginosa* (from Dr Homma of Tokyo University, Institute of Medical Science), who also provided strains p28, c18 and K. The FP⁺ strain was obtained from Dr T. Watanabe of Keio University, and other strains used in these experiments were isolated from patients at Kyushu University Hospital. All the strains were tested for the production of pyocin, rhabidosomes and phages.

Media and cultivation of bacteria. Bacteria were grown in peptone broth or heart infusion broth (Difco) with aeration. GS medium, a synthetic medium, contained (g./l.): $(\text{NH}_4)_2\text{HPO}_4$, 2.5 g; KH_2PO_4 , 1.5; NaCl, 5.0; sodium glutamate, 3.0; glucose, 3.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; CaCl_2 , 0.05; yeast extract, 1.0; pH 7.2.

For induction, mitomycin C (Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan) was added to the culture in logarithmic phase at a final concentration of $3 \mu\text{g./ml.}$ In experiments at temperatures other than 37° , cells growing logarithmically at 37° were held at the designated temperature for 1 h. and then induced by mitomycin.

Antisera and micro-agglutination technique. Antisera against pyocin were prepared as follows: Pyocin induced from strain 3R at 25° was partially purified by differential centrifugation, then, mixed with Freund's adjuvant, it was injected intradermally into rabbits. Two weeks later a second injection was given without adjuvant, and 10 days later blood was collected by heart puncture and serum was prepared. Antibodies against the components of the host cell were adsorbed with large amounts of host cells prior to use. The micro-agglutination technique of Hummeler, Anderson & Brown, (1962) was used: serum was diluted ten times with phosphate-buffered saline (pH 7.0) and mixed with an equal volume of concentrated pyocin prepared by concentrating the crude lysate of strain 3R by ultracentrifuging at 30,000 rev./min. for 1 h.; it contained R-type and 28-type pyocins and rhabidosomes. After standing at room temperature for 30 min., the mixture was centrifuged at 10,000 rev./min. for 30 min. The resulting pellets were resuspended in a small volume of 2% ammonium acetate solution and examined under the electron microscope.

Electron microscopy. Mitomycin-induced lysate was treated with desoxyribonuclease to reduce the viscosity due to host cell DNA, then after low-speed centrifugation to remove cell debris, pyocin and rhabidosomes were centrifuged down at 30,000 rev./min. for 1 h. Pellets were resuspended in 2% ammonium acetate solution and the suspension was negatively stained with neutral potassium phosphotungstate.

RESULTS

Effects of mitomycin C on the growth of strain 3R. To logarithmically growing cells at 37° $3 \mu\text{g.}$ of mitomycin C/ml. were added and cell growth was followed in terms of optical density at 660 nm. For 1 h. after induction density of the culture increased parallel to that of the control, and then decreased rapidly (Fig. 1).

Morphology of pyocin and rhabidosomes. Strain 3R produced two types of pyocin (Pl. 1, fig. 1). One, pyocin 28, had a structure similar to that of λ phage tails (Takeya, Minamishima, Amako & Ohnishi, 1967) and the other, pyocin R, resembled T-even phage tails (Ishii, Nishi & Egami, 1965). R-type pyocin had a contractile sheath with a diameter of 150 Å in the extended, and 180 Å in the contracted, state. Rhabidosomes were long rods 180 Å in diameter (Pl. 1, fig. 2, 3). Since phosphotungstate penetrated into the rhabidosomes they are thought to be hollow, of internal diameter about 80 Å. Their granular appearance suggests that they are constructed of small irregularly arranged subunits (Pl. 1, fig. 3).

Morphologically they resemble the polysheath of a T-even phage and might be polymerized sheath proteins of R-type pyocin.

Antigenic similarity of R-type pyocin and rhabidosomes. The suggestion of the last paragraph was investigated by specific micro-agglutination, as revealed by the electron

microscope (Hummeler *et al.* 1962). It was found that R-type and 28-type pyocin formed specific agglutinates, and that rhapidosomes agglutinated with R-type, but not with 28-type pyocins (Pl. 2, fig. 4, 5; Pl. 3, fig. 6). Antibody bridges were clearly seen between rhapidosome and R-type pyocin (Pl. 2, fig. 5 arrow) indicating antigenic similarity of the two.

Fig. 1. Effect of mitomycin C on the growth of *Pseudomonas fluorescens* strain 3R at 37°. Mitomycin C was added to the logarithmic phase of the culture at a final concentration of 3 $\mu\text{g./ml.}$ Growth was followed by observing changes in extinction (E) at 660 nm. Control, $\bigcirc$ — $\bigcirc$; mitomycin-treated, $\bullet$ — $\bullet$.

Conditions for rhapidosome production. The 3R strain was able to grow even in synthetic medium and to be lysed by mitomycin. Rhapidosome and pyocin production under various conditions is summarized in Table 1. When induction took place at low temperature (25°), 5 h. or more were required for complete cell lysis (Fig. 2) and no rhapidosomes were produced.

The lysate obtained at 40° contained small ring structures about 120 Å in diameter (Pl. 3, fig. 7) as well as short rods which might be polymerized forms of these rings.

Production of rhapidosomes in several strains of the Pseudomonas group. Data obtained so far suggest that the rhapidosome released from strain 3R is a polymerized

sheath protein of R-type pyocin. Accordingly, the relationship of rhabidosome and R-type pyocin production was investigated in several strains of the *Pseudomonas* group. Sixteen strains were induced with mitomycin C, the lysates were negatively stained, examined under the electron microscope, and the presence or absence of R-type and 28-type pyocin, rhabidosomes and phages was recorded.

Table 1. *Production of rhabidosomes and pyocin by mitomycin induction from Pseudomonas fluorescens strain 3R under various conditions*

Medium	Temperature	Cell growth	Pyocin production	Rhabidosome production
Heart infusion broth	40°	+	+	+
	37°	++	++	++
	28°	++	++	++
	25°	+	++	—
Peptone broth	37°	++	++	+
Synthetic medium (CS)	37°	+	++	+

Table 2. *Production of pyocin and rhabidosomes from several strains of the Pseudomonas group*

Strain	28-Type pyocin	R-type pyocin	Rhabidosome	Phage*
FP ⁺	+	+	+	—
3R	+	+	+	—
K	+	+	+	—
118	+	+	+	+
A 31	+	+	+	—
37	+	+	+	—
67	+	+	+	+
B 10	+	+	+	—
A 22	+	+	—	—
22	—	+	+	—
B 62	—	+	—	—
C 18	+	—	—	+
P 28	+	—	—	—
A 30	+	—	—	—
B 46	+	—	—	—
C 14	+	—	—	+
B 52	—	—	—	+

* All phages induced from these strains are of λ phage type.

As shown in Table 2 rhabidosomes were produced concomitantly with R-type pyocin but not with 28-type pyocin. Only in two strains (A 22, B 62) which formed R-type pyocin could no rhabidosomes be seen, but as they were often scanty it is possible that they were missed. Phages induced in these strains all had the structure of λ phage.

DISCUSSION

It was concluded from these experiments that the rhabidosome induced from *Pseudomonas* is polymerized sheath protein of R-type pyocin. Morphologically it has the structure of the contractile sheath of the pyocin and it also resembles the polysheath which appeared in the lysate of a conditionally lethal mutant of T-even

phage (Epstein *et al.* 1963). Rhapidosomes always have the same diameter as the contracted, not extended, sheath. The polysheath of the T4 mutant is the polymerized sheath protein of the phage tail and also has the structure of the contracted sheath (Kellenberger & Boy de la Tour, 1964). Our micro-agglutination experiments showed that the rhapidosome and R-type pyocin have the same antigenicity, which supports the idea that the rhapidosome is made from the same protein as the sheath of R-type pyocin.

Fig. 2. Effect of mitomycin C on the growth of *P. fluorescens* strain 3R at 25°. Control, ○—○; mitomycin-treated, ●—●.

In spontaneous lysates of older cultures of strain 3R no rhapidosomal structures were detected (cf. Lewin, 1963; Yamamoto, 1967), but they were readily found in mitomycin-induced lysates. Several strains of *Pseudomonas aeruginosa* or *P. fluorescens* induced by mitomycin produced rhapidosomes together with R-type but not with 28-type pyocin. Correll & Lewin (1964) analysed purified rhapidosomes obtained from *Saprospira* and showed that they too were uniform in size with a constant *S* value and consisted of RNA and protein.

At present all rod-shaped particles released from bacteria are called rhapidosomes, and from these observations it is clear that there are at least two types. However, the rhapidosomal structure whose origin has been proved to be phage-tail or bacteriocin should be called 'polysheath' and not 'rhapidosome'.

The ring structure observed in the lysate at 40° might be the sheath protein assembled into the ring form or a short rod. Why it is not assembled into complete pyocin has not yet been investigated.

To obtain more direct evidence for the mechanism of polysheath formation we are now trying to isolate sheath protein and reassemble it into polysheaths *in vitro*.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Pyocin induced from the 3R strain of *Pseudomonas fluorescens*. Two types of pyocin can be seen. One, R-type (R), has a structure like T-even phage-tails; the other, 28-type (28), resembles λ phage-tail. R-type pyocin has a contractile sheath. Negatively stained with neutral potassium phosphotungstate (PT). Scale marker represents 100 nm.

Fig. 2. Rhabidosome from strain 3R.

Fig. 3. Rhabidosomes and R-type pyocin from strain 3R. Rhabidosomes (RH) are long rods 180 Å in diameter. Their surface looks granular. Note penetration of PT into the rods.

PLATE 2

Fig. 4, 5. Micro-agglutination between rhabidosomes and pyocins. R-type pyocins and 28-type pyocins form different specific agglutinates. Rhabidosomes agglutinate together with R-type pyocins. Antibody bridges can be seen between pyocin rods and rhabidosomes (arrow).

PLATE 3

Fig. 6. Micro-agglutination between rhabidosomes and pyocin. R-type and 28-type pyocin form different agglutinates.

Fig. 7. Lysate from strain 3R induced at 40°. A large number of small spherical particles can be seen, some of them arranged into short rods (arrows).

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