MODE OF ACTION OF PYOCIN: INACTIVATION OF RIBOSOMES IN SUPPORTING POLY U DIRECTED INCORPORATION OF PHENYLALANINE

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Pyocin, a bacteriocin of Pseudomonas aeruginosa, was first described by Jacob (1954) who showed that pyocin, like colicin, exerted a bacteriocidal effect on the sensitive cells. Kageyama and Egami (1962) and Kageyama (1964) have recently succeeded in purifying a pyocin from the lysate of UV- or mitomycin C induced cells of P. aeruginosa R (obtained from Dr. Y. Homma of the Institute for Infectious Diseases, University of Tokyo). The purified pyocin was homogeneous in ultracentrifugation and its molecular weight was calculated to be around 1×10^7.

The mode of action of bacteriocins has been studied by several groups of investigators. The apparent mode of action seems to be different depending on the kind of bacteriocins studied. Thus, colicin ML (Jacob et al., 1952) and colicin K (Nomura and Nakamura, 1962) were shown to inhibit the synthesis of DNA, RNA, and protein, while colicin E_3 seems to affect only protein synthesis (Nomura, 1963). Colicin E_2 was found to provoke the degradation of DNA of the sensitive cells in addition to the inhibition of the synthesis of RNA and protein. A similar observation on DNA degradation was reported with megacin C by Holland (1963).

The addition of pyocin to sensitive cells was found to result in the immediate cessation of DNA, RNA, and protein synthesis as measured

by the incorporation of C^{14}-adenine and C^{14}-phenylalanine into the TCA-insoluble fraction (Kaziro and Tanaka, in preparation). Since the incorporation of P_i^{32} into the TCA-soluble fraction was depressed to a much smaller extent, and the respiration decreased rather gradually for a long period of time (Kaziro and Kameyama, unpublished observations), the energy metabolism is not likely to be the primary site of the pyocin action. In the present communication, we wish to present some evidence which demonstrates that ribosomes isolated from pyocin-treated cells are no longer able to carry out poly U-directed incorporation of C^{14}-phenylalanine into TCA-insoluble fraction.

P. aeruginosa K (pyocin-sensitive strain) was grown in 200 ml of glutamate-glucose media (Kageyama and Egami, 1962) to 2 x 10^9 cells per ml. Pyocin, 0.6 μg per ml of culture, was added, and the incubation was continued for another 15 minutes to ensure adsorption on the sensitive cells. A viable count assay performed at this point gave a survival of 3.8%. The culture was rapidly cooled in ice and the cells were collected by centrifugation for 5 minutes at 12,000g, washed twice, and disrupted by sonication. The extracts were centrifuged twice at 17,000g for 30 minutes and the supernatant (S-17) was used for C^{14}-phenylalanine incorporation experiments. A control sample was run in exactly the same way without addition of pyocin.

Table I shows that S-17 fraction obtained from pyocin-treated cells had little activity in incorporating C^{14}-phenylalanine into the hot TCA-insoluble fraction in the presence of poly U and an ATP-GTP regenerating system. The results show at the same time that in P. aeruginosa, a bacteria with DNA of high GC content (AT/GC=0.49, Belozersky and Spirin, 1958), poly U directs the incorporation of phenylalanine, an observation which is in agreement with that of Protass et al. (1964) with Alcaligenes faecalis. Experiment 3 of Table I indicates that in the tube which contained extracts both from pyocin-treated and non-treated cells, only the activity of non-treated cells

Table I

Incorporation of C^{14}-phenylalanine by S-17 fraction.

The reaction mixture contained in μmoles: Tris-HCl, pH 8.0, 25; NH_4Cl, 17; $MgSO_4$, 3.5; mercaptoethanol, 1.5; ATP, 0.25; GTP, 0.025; creatine phosphate, 2.5; creatine kinase, 20μg; sRNA, 750μg; C^{14}-phenylalanine(5μc/μmole), 0.02; poly U, 20μg; and S-17 fractions as indicated in a final volume of 0.25ml. After incubation for 30 minutes at 37°, the radioactivity incorporated into TCA precipitates was measured according to the procedure of Zamecnik et al. (1951).

Expt.	System	Incorporation
		cpm.
1.	Normal(580μg) Complete system	1,404
	-poly U	50
2.	Pyocin-treated(580μg) Complete system	64
	-poly U	41
3.	Normal(290μg) and pyocin-treated(290μg) Complete system	750
	-poly U	40
4.	Normal(720μg)* Complete system	1,173
	-poly U	74
	+pyocin(0.12μg)	1,155

* A different preparation of S-17 fraction.

Table II

Activity of phenylalanyl-sRNA synthetase

The assay of the enzyme activity was carried out essentially in the same way as described by Berg et al. (1961) with 0.1μmole of C^{14}-phenylalanine(2.5μc/μmole), 750μg of sRNA, and S-17 fractions with 1.15 mg of protein.

System	Formation of C^{14}-phenylalanyl-sRNA
	cpm/mg protein/15 minutes
S-17 from normal cells	1,730
S-17 from pyocin-treated cells	1,300

was manifested. This suggests that there is no accumulation of free inhibitor in the extracts of treated microorganism. The addition of pyocin to the in vitro system (Expt. 4) was not inhibitory to

polyphenylalanine synthesis.

The assay of the phenylalanyl-sRNA synthetase revealed (Table II) that the activity of the enzyme was only slightly decreased in pyocin-treated cell extracts as compared with that from non-treated cells. Therefore one could assume that the inhibition of protein synthesis occurred at the step(s) somewhere later than aminoacyl-sRNA formation.

The S-17 fraction was centrifuged at 105,000g for 2 hours to give the supernatant(S-100) and the precipitate(ribosome) fractions. The results in Table III demonstrate that the ribosomes of the pyocin-treated cells are inactive in poly U-dependent polyphenylalanine synthesis when incubated with the S-100 fraction from non-treated cells,

Table III

Incorporation of C^{14}-phenylalanine by ribosomes and S-100 fraction.

The composition of the reaction mixture and the procedure for the assay was essentially same as described in Table I. The preparations contained: S-100(normal and pyocin-treated), 234μg of protein; ribosome (normal), 268μg of RNA; and ribosome(pyocin-treated), 235μg of RNA.

ribosome	S-100	C^{14}-phenylalanine incorporated	
		without poly U	with poly U
		cpm.	cpm.
normal	normal	31	1,267
pyocin-treated	pyocin-treated	30	88
normal	pyocin-treated	29	989
pyocin-treated	normal	39	68

whereas, the S-100 fraction from the treated cells is almost as active as that from non-treated cells in combination with normal ribosomes. An experiment not shown in the table demonstrated that the results were essentially similar in the absence of added sRNA although the incorporation of the labelled amino acid was about one-half to one-third of that in the presence of added sRNA.

Discussions. From the foregoing results, it appears that the cessation of protein synthesis provoked by adsorption of pyocin is probably due to inactivation of the ribosomes. The detailed mechanism of this inactivation is not clear. Since pyocin, when added to the in vitro system, is not inhibitory, it is not likely that the observed inactivation involves the direct binding of pyocin to the ribosomes, although the possibility that some components of pyocin enter the cells to interact with ribosomes, is not fully excluded. From the experiment in which the S-17 fractions of both pyocin-treated and normal microorganisms were mixed, it may be deduced that there is no accumulation of free inhibitor, either external or internal, in the extracts of the treated cells.

The results were rather unexpected and, at present, it is difficult to visualize any detailed mechanism by which, upon adsorption of a large protein molecule onto the surface of the bacteria, even pre-existing ribosomes become unable to participate actively in protein synthesis. The clarification of this point must await further investigation. It must also be pointed out that the inactivation of ribosomes might not be the primary site of pyocin action, since we have not yet determined the activity of RNA- and DNA nucleotidyl transferase in extracts of the pyocin-treated microorganisms. The studies now under way on the physicochemical properties of the ribosomes isolated from pyocin-treated cells may give us further insight on the nature of the observations reported here.

Conclusion. Pyocin, a bacteriocin of Pseudomonas aeruginosa was shown in inhibit RNA, DNA, and protein synthesis following its adsorption on the surface of the sensitive bacteria. The extracts from the pyocin-treated cells were unable to carry out the poly U dependent incorporation of C^{14}-phenylalanine. It was found that this inhibition is due to inactivation of the ribosome fraction. The activity of phenylalanyl-sRNA synthetase was not affected appreciably.

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