Mutants of *Pseudomonas aeruginosa* with Impaired β -Lactamase Inducibility and Increased Sensitivity to β -Lactam Antibiotics

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(Received 1 January 1973)

INTRODUCTION

The first systematic investigation of the inducible β -lactamase of *Pseudomonas aeruginosa* was carried out by Sabath, Jago & Abraham (1965). All strains of *P. aeruginosa* examined so far synthesize inducible β -lactamases that are indistinguishable from one another and require high concentrations of penicillin G for optimal induction (Sabath *et al.* 1965; Garber & Friedman, 1970; Bobrowski & Borowski, 1971; Sykes & Richmond, 1971). The purified enzyme is defined as moderate cephalosporinase (β -lactamase class Id) (Richmond & Sykes, 1972) and is inhibited by carbenicillin, cloxacillin and methicillin (Bobrowski & Borowski, 1971; Richmond & Sykes, 1972). The efficacy of carbenicillin (Acred *et al.* 1967) against *P. aeruginosa* seems at least to some extent to depend on its stability against the 'Sabath & Abraham' (S & A) enzyme. Nevertheless, different strains of *P. aeruginosa* vary greatly in their response to carbenicillin, the minimal inhibitory concentrations (m.i.c.) of which range from 5 to > 500 μ g/ml (Silverblatt & Turck, 1969).

This communication deals with the preparation of mutants of *Pseudomonas aeruginosa* severely impaired in inducibility of the S & A β -lactamase and describes their sensitivities to different penicillins and to cephaloridine in comparison with that of the corresponding wild-types.

METHODS

Pseudomonas aeruginosa strains 2126 (Newsom, Sykes & Richmond, 1970), McPhilipps (Professor M. H. Richmond, Bristol) and ATCC 12055 were used in this study. They were grown exponentially in brain-heart infusion broth and mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (20 μ g/ml, 4 min, 37 °C). After segregation, the organisms were plated on brain-heart infusion agar containing 0.5 % starch and incubated overnight at 37 °C. The resultant colonies were covered with agar containing 6-amino penicillanic acid (6-APA) to give a final inducing concentration of 400 μ g/ml. 6-APA was chosen as inducer on account of its great inducing power coupled with its marked stability against the S & A enzyme (Garber & Friedman, 1970), whereas carbenicillin has the disadvantage of being an inhibitor. After 5 h induction at 37 °C the plates were treated with a solution of 1 % penicillin G in 0.067 M phosphate buffer at pH 7.0 containing I_2/KI (0.008 M/0.06 M). Colonies developing no white haloes indicative of β -lactamase production were picked as putative S & A negatives. They were checked for inducible β -lactamase production as described in the legend of Table 1.

Table 1. β -Lactamase production and sensitivity to β -lactam antibiotics of mutants of *Pseudomonas aeruginosa* defective in the induction mechanism in comparison with the corresponding figures of the wild-types

CY broth (Jack & Richmond, 1970) was inoculated to a density of 10^8 bacteria/ml. Cultures were induced by shaking for 3 h at 37 °C in the presence of 6-APA at concentrations of 50 μ g/ml for strains 2126 and ATCC 12055 and at 25 μ g/ml for the McPhilipps strain. Uninduced controls were run in parallel. Both induced and uninduced cultures reached densities of approximately 5×10^9 bacteria/ml. After centrifugation, bacteria were resuspended in phosphate buffer 0.067 M, pH 7.0, and smashed in a Branson sonifier under refrigeration. The homogenates (20 to 60 μ l) were added to 2 ml 10^{-4} M-cephalothin kept at a constant temperature of 37 °C. The decrease in $E_{260\text{nm}}$ was recorded in a spectrophotometer (O'Callaghan, Muggleton & Ross, 1969). β -Lactamase activity was then expressed as the difference in $E_{260\text{nm}}$ /h in relation to the biomass of the culture (mg dry weight/ml). All activities were completely inhibited by 10^{-4} M-carbenicillin.

Strain no.	β -Lactamase activity		Induction factor*	Minimal inhibitory concentrations (m.i.c.)† (μ g/ml)				Cephaloridine
	-induction	+induction		6-Amino-penicillanic acid	Penicillin G	Ampicillin	Carbenicillin	
2126-WT	0.48	18.0	37.5	550	> 1000	250	40	> 1000
2126-8	0.47	0.81	1.7	250	70	30	50	30
ATCC 12055-WT	0.26	20.3	78.1	220	500	40	20	> 1000
ATCC 12055-3	0.20	0.68	3.4	60	30	6	15	20
McPhilipps-WT	0.47	83.3	177.2	30	100	5	0.4	> 1000
McPhilipps-5	0.27	0.20	0.74	15	3	0.4	0.5	5

* The induction factor represents the induced β -lactamase level as a multiple of the basal activity.

† The m.i.c. were determined on brain-heart infusion agar containing a gradient of the test antibiotic. Cultures with 10^7 bacteria/ml were streaked on the gradient. The m.i.c. were read after overnight incubation at 37 °C. The antibiotics were obtained from commercial sources.

RESULTS AND DISCUSSION

Approximately 10^4 colonies were screened per strain, yielding 11 β -lactamase-negative mutants, i.e. three for strain 2126 and four each for the McPhilipps and ATCC 12055 strains. The same number of colonies were screened without induction for constitutive β -lactamase synthesis but no such mutants were found under these conditions.

Table 1 shows the inducibility by 6-APA of the S & A enzyme in the wild-types and one representative mutant per strain. All mutants produced similar basal β -lactamase levels to the wild-types and were either completely inert to induction or at least severely impaired in their inducibility. The induction factors of the 11 mutants ranged from 0.7 to 8.1. The mutants may therefore be affected in the hypothetical repressor of the β -lactamase operon, its affinity for the inducer being abolished or considerably weakened. The fact that no completely β -lactamaseless mutants were found suggests that this enzyme may play a vital physiological role in addition to its protective function against β -lactam drugs.

As indicated in Table 1, the loss of inducibility results in a dramatic decrease in the m.i.c. of penicillin G, ampicillin and cephaloridine, whereas sensitivity to carbenicillin and 6-APA remains virtually unchanged. This reveals the stability of 6-APA and the absolute inertness of carbenicillin towards the hydrolytic activity of the S & A enzyme (Garber & Friedman, 1970; Bobrowski & Borowski, 1971; Richmond & Sykes, 1972) and shows that stability against this enzyme is necessary (though not the only prerequisite) to enable β -lactam drugs to act efficiently on *Pseudomonas aeruginosa*.

The wide range of response to the action of β -lactam antibiotics shown by the wild-types is reflected in the intrinsic sensitivity or resistance of the induction-negative mutants. Quite

obviously, there are resistance mechanisms other than β -lactamase production (Richmond & Sykes, 1972).

The disparity in the sensitivity of the three strains may well be due to differences in their permeability parameters. Since permeability barriers obviously reduce both the antibacterial concentrations at the target site and the intracellular inducer content, one would expect the inducibility and the sensitivity of a strain to be correlated. Although they are only conclusive in respect of 6-APA, the data in Table 1 lend support to this assumption: strain 2126, which is highly resistant to 6-APA, is induced by 50 $\mu\text{g/ml}$ to a much less extent than is the much more sensitive McPhilipps strain by 25 $\mu\text{g/ml}$. Strain ATCC 12055 is intermediate both in its sensitivity and its inducibility.

We are grateful to Professor M. H. Richmond and N. Curtis for helpful discussions.

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