WILD TYPE VARIANTS OF PENICILLINASE FROM *KLEBSIELLA AEROGENES*

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1. Introduction

Exopenicillinase from *Staphylococcus aureus* and *Bacillus licheniformis* have been shown [1,2] to exist in, respectively, three and two closely related but distinctly different forms. This phenomenon has been termed "wild type variation". Experiments described in the present paper suggest that such variation may also exist in *Klebsiella aerogenes,* in respect of two enzymatic properties, substrate specificity patterns and relative affinities for inhibitors.

2. Methods and materials

The eleven strains of *K. aerogenes,* used in previous studies [3], were investigated: laboratory reference numbers 1, 43, 83, 366, 370, 373, 402, 407, 414, 415 and 418. Organisms were cultured in infusion broth at 37° overnight, harvested by centrifugation, resuspended in 25 mM sodium phosphate buffer pH 7.4, and disrupted by treatment for 5 minutes in a 60 watt ultrasonic disintegrator. Penicillinase activity was measured by the hydroxylamine method [4]. Substrate specificity profiles were determined by measuring the rates at which the β-lactam ring of the following compounds was hydrolysed, relative to that of benzylpenicillin: phenoxymethylpenicillin, phenethicillin, propicillin, phenbenicillin, ampicillin and 6-aminopenicillanic acid. The affinities of methicillin, quinacillin and cloxacillin, relative to that of benzylpenicillin, and activation energies and pH optima for each of the penicillinase preparations were determined as previously described [5,6].

Table 1
Substrate specificity patterns of *K. aerogenes* penicillinase preparations. Rates of hydrolysis are expressed in terms of rate of benzylpenicillin hydrolysis for each strain (= 100).

Strain of *K. aerogenes*	Phenoxymethyl-penicillin	Phenethicillin	Propicillin	Phenbenicillin	Ampicillin	6-Amino-penicillanic acid
1	117	42	16	21	211	60
43	151	74	61	59	233	101
83	158	84	38	58	163	41
366	117	43	15	18	198	73
370	124	55	23	25	188	60
373	118	43	16	19	195	77
402	110	43	19	25	228	68
407	113	44	18	24	185	55
414	106	43	13	18	152	48
415	143	48	16	22	262	77
418	169	72	36	47	175	55

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3. Results

Table 1 shows the rates of hydrolysis of the six substrates relative to that of benzylpenicillin; each figure is the mean of at least three determinations. An examination of these figures suggested that the preparations from three of the strains (43, 83 and 418) hydrolysed phenoxymethylpenicillin, phenethicillin, propicillin and phenbenicillin more rapidly, relative to the rate of hydrolysis of benzylpenicillin, than did the other preparations. A statistical analysis, summarized in table 2, shows that the differences between the relative rates of hydrolysis of the four phenoxypenicillins by the two groups are highly significant; ampicillin and 6-aminopenicillanic acid are not hydrolysed at significantly different rates by the two groups. The coefficient of variation, V (= S.D./mean), for each of the results shown in table 2 is considerably in excess of that observed for the assay method itself ($V = 0.036$, for a series of ten identical samples), and thus it seems probable that the observed scatter in the results shown in table 2 is due to "biological variation" rather than to errors arising from the method of assay.

Values of the relative affinities of methicillin, quinacillin and cloxacillin are shown in table 3. It is apparent that the enzyme from strain 83 behaves differently from the others, in that K_i/K_m values for all three inhibitors with this strain exceed the mean values for the other strains by 3 × S.D., or more.

There was no significant difference between the activation energies for benzylpenicillin hydrolysis for the two groups; values obtained were – for the "majority" group 3.3 cal/mole, S.D. 0.4; for the "minority" group 3.8 cal/mole, S.D. 0.5. $t = 1.56$, giving $0.2 > P > 0.1$ for nine degrees of freedom.

All the preparations studied had a wide range of pH over which their activities were close to maximal; pH optima were about 7.0, and there were no well-defined differences between the groups.

4. Discussion

The properties of the enzymes studied here support the idea that there are two populations (of eight and three strains, respectively) of *K. aerogenes* in respect of the substrate specificity patterns of their penicillinases; in terms of pH optima and activation energies, the enzymes seem to belong to one homogeneous population. Ten of the enzymes show a range of K_i/K_m values that appear to be normally distributed, but the remaining strain (83) has an enzyme for which the values of K_i/K_m exceed the

Table 2

Statistical analysis of substrate specificity patterns from two groups of *K. aerogenes*.

	Mean relative rates of substrate hydrolysis ± S.D.			
	Strains 1, 366, 370, 373, 402, 407, 414, 415	Strains 43, 83, 418	*t* value	Significance
Phenoxymethylpenicillin	118.5 ± 11.3	159.3 ± 9.1	5.556	
Phenethicillin	45.1 ± 4.4	76.7 ± 6.4	9.465	Highly
Propicillin	17.0 ± 3.0	45.0 ± 13.9	5.849	significant
Phenbenicillin	21.5 ± 3.0	54.7 ± 6.7	8.351	($P < 0.001$)
Ampicillin	202.4 ± 32.5	190.3 ± 11.8	0.1438	Not
6-Aminopenicillanic acid	64.8 ± 10.7	65.7 ± 9.9	0.0766	significant ($P > 0.8$)

Table 3
Values of K_i/K_m, using benzylpenicillin as substrate, for penicillinase preparations from *K. aerogenes* strains, determined for three inhibitors.

Strain *K. aerogenes*	Competitive inhibitor		
	Methicillin	Quinacillin	Cloxacillin
1	0.00417	0.077	0.446
43	0.0055	0.101	0.471
83	0.0636	0.249	0.65
366	0.00274	0.0414	0.242
370	0.0045	0.082	0.375
373	0.00232	0.0434	0.271
402	0.00595	0.082	0.403
407	0.00725	0.1	0.437
414	0.0061	0.106	0.476
415	0.00546	0.0846	0.368
418	0.00497	0.0744	0.368
Mean) excluding	0.0049	0.079	0.386
S.D.) strain 83	0.0015	0.022	0.079

mean values for the other enzymes by 3 × S.D., or more. Such a departure from the mean value would be expected in about one case in four hundred in a normally distributed population. While it is not possible to be certain at this stage that the enzyme from strain 83 is in fact different from the others in respect of its K_i/K_m values, it seems highly likely that it is.

An analysis of resistance to hydrolysable penicillins (from data given in [3]) showed that there were no significant differences in relative resistance between the two groups; this finding is of interest in view of the observed difference in relative rates of hydrolysis, and adds further support to the idea [3] that β-lactamase plays only an incidentical role in penicillin-resistance in *Klebsiella.*

The β-lactamase from *K. ozaenae* 61 [3] was also examined, and its properties were found to resemble closely those of the "majority" group of *K. aerogenes*

5. Conclusion

A consideration of substrate specificity and affinity for competitive inhibitors reveals three closely related but distinct variants of β-lactamase in a population of twelve strains of *Klebsiella.* If the substrate specificity pattern and the affinity pattern of the enzymes from the "majority" group (*K. aerogenes* 1, 366, 370, 373, 402, 407, 414 and 415 and *K. ozaenae* 61) are described as S_1 and A_1 respectively, the substrate specificity pattern of the enzymes from the "minority" group (*K. aerogenes* 43, 83 and 418) as S_2, and the affinity pattern of the enzyme from strain 83 as A_2, the three enzyme types may be designated thus:

type I S_1A_1 strains 1, 366, 370, 373, 402, 407, 414, 415 and 61.
type II S_2A_1 strains 43 and 418.
type III S_2A_2 strain 83.

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