A Direct Comparison of Two Unusual R-Factor-Mediated β -Lactamases

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(Received 21 February 1972)

The resistance factor R-1818 (also known as R-46), originally isolated in Dr. E. S. Anderson's laboratory, mediates the production of a β -lactamase (penicillin/cephalosporin β -lactam amidohydrolase, EC 3.5.2.6) that is unusual in several respects. It hydrolyses oxacillin, which is normally regarded as a β -lactamase-resistant penicillin, about six times as rapidly as benzylpenicillin. It is inhibited by Cl^- ions. Its molecular weight (44 600) is much larger than that of other β -lactamases so far described (Smith, 1969; Dale & Smith, 1971a; Dale, 1971). This enzyme has been purified and its properties have been investigated in some detail (Dale & Smith, 1971a; Dale, 1971). Yamagishi *et al.* (1969) had purified and characterized a β -lactamase specified by the R-factor R-GN238, which was also active against certain isoxazolympenicillins and inhibited by Cl^- ions. Dale (1971), by comparing the properties of the R-GN238-mediated β -lactamase as described by Yamagishi *et al.* (1969) with those obtained for R-1818-mediated β -lactamase (Dale & Smith, 1971a; Dale, 1971), concluded that although they were probably closely related they did not appear to be identical. However, at that stage they had not been examined side by side in one laboratory. A direct comparison was therefore necessary to establish the relationship between these two enzymes, which is of interest with regard to the evolution of R-factors and β -lactamases. Dr. Sawai has kindly sent us a strain of *Escherichia coli* harbouring R-GN238, so enabling a direct comparison of the properties of the two β -lactamases to be made in this laboratory.

To eliminate the possibility of host-specified differences, it was desirable to transfer the R-factor to one of the strains of *Escherichia coli* K 12 used in this laboratory as host for the studies previously made with resistance factor R-1818. Resistance factor R-GN238 was therefore transferred from the strain in which it was received, *E. coli* W 3630 (R-GN238) (*mal*⁻), to *E. coli* J 6-2 (*trp*⁻, *his*⁻, *pro*⁻, *lac*⁻) by selection on minimal agar (Davis & Mingioli, 1950) lacking citrate and containing maltose as sole carbon source. Streptomycin (10 $\mu\text{g}/\text{ml}$) was used to select for strain J 6-2 carrying resistance factor R-GN238 and the mating was carried out by the method of Smith (1969). Transfer occurred at a frequency of 6×10^{-2} per input donor. The *lac*⁻ nature of the recipient was confirmed on MacConkey agar, and the presence of the transferred R-factor by testing for antibiotic

resistances. It was found that single-cell inocula grew unrestricted on ampicillin, chloramphenicol or streptomycin (50 $\mu\text{g}/\text{ml}$) or on tetracycline (10 $\mu\text{g}/\text{ml}$) in Davis-Mingioli agar. All subsequent work was done with crude preparations of β -lactamase from this *E. coli* J 6-2 (R-GN238). The methods used are described by Dale & Smith (1971a).

The molecular weight of R-GN238-mediated β -lactamase, determined by chromatography on a column of Sephadex G-100, was found to be 23 300, which is similar to the molecular weights of many other β -lactamases found in Gram-negative bacteria (Dale & Smith, 1971b). It is notable, however, that this value is very much lower than the value of 44 600 obtained for the molecular weight of the R-1818-mediated enzyme (Dale, 1971). In addition, the apparent molecular weight of the R-GN238-mediated β -lactamase was found to be unaffected by the presence of Dextran Blue, whereas the R-1818-mediated enzyme bound to it and was eluted near the void volume in its presence (Dale, 1971).

The Cl^- ion-sensitivity, however, was similar for the two enzymes: 50% inhibition occurred at 16 mM for both the R-1818-mediated and the R-GN238-mediated β -lactamase, which agrees with previously published results (Yamagishi *et al.*, 1969; Dale, 1971).

The β -lactamase specified by resistance factor R-GN238 was considerably more temperature-sensitive than was the enzyme mediated by resistance factor R-1818. The former lost 50% of its activity in 6 min at 45°C, whereas 25 min at 45°C was required for 50% inactivation of the latter, at the same concentration of cell extract. When this experiment was repeated with enzyme preparations that had been diluted to similar concentrations of β -lactamase activity, essentially the same results were obtained. Hence it can be concluded that the greater sensitivity of the R-GN238-mediated β -lactamase to heat cannot be due to the lower enzyme concentration mediated by the R-factor, but is due to a difference in the enzyme protein.

The relative activities and K_m values of the two enzymes for a number of substrates were determined, and are compared in Table 1. It is significant that the R-GN238-mediated enzyme has a high activity against methicillin (another 'penicillinase-stable' penicillin), and a high affinity for the isoxazolympenicillins, when compared with the R-1818-mediated β -lactamase. [It should be noted that the K_m value

Table 1. *Substrate specificity of the R-1818-mediated β -lactamase and the R-GN238-mediated β -lactamase*

Relative activities were determined at pH 7.4 and 30°C by using the hydroxylamine assay (Dale & Smith, 1971a); the substrate concentration was 2 mg/ml (1 mg/ml for 6-aminopenicillanic acid). K_m values were determined at pH 7.4 and room temperature (approx. 20°C) by using the micro-iodometric assay (Novick, 1962; Dale & Smith, 1971a). Results in parentheses are those obtained by Yamagishi *et al.* (1969).

β -Lactamase mediated by resistance factor ...	Relative activity (V_{max}) (units/ml)		K_m (μM)	
	R-1818	R-GN238	R-1818	R-GN238
Benzylpenicillin	100	100	4	2 (5)
Penicillin V (phenoxymethylpenicillin)	188	118	15	2
Phenethicillin (phenoxyethylpenicillin)	387	155	16	4 (7)
Propicillin (phenoxypropylpenicillin)	557	226	20	23
Ampicillin	175	376	6	12 (18)
6-Aminopenicillanic acid (sodium salt)	68	19	60	— (26)
Methicillin	28	306	—	13
Cloxacillin	201	108	230	3 (15)
Oxacillin	667	185	90	2
Cephalexidine	43*	29*	60	— (111)

* Because of the unsuitability of the standard hydroxylamine assay with cephalosporins, a slightly modified assay was used (Dale & Smith, 1971a).

for cloxacillin obtained with the R-1818-mediated enzyme is only approximate, owing to the difficulties of using the micro-iodometric assay at high substrate concentrations (Dale, 1971).] Of the substrates tested, the one most rapidly hydrolysed by the R-1818-mediated enzyme, when saturated, was oxacillin, whereas the R-GN238-mediated enzyme was most active against ampicillin under these conditions. With regard to the affinity of the enzymes for β -lactam antibiotics, although the R-1818-mediated β -lactamase exhibited a high affinity for only benzylpenicillin and ampicillin, the R-GN238-mediated enzyme has a high affinity for a broad range of substances. Table 1 also shows that, with the possible exception of cloxacillin, there is a good agreement between the results obtained by Yamagishi *et al.* (1969) with those reported here for the K_m values of the β -lactamase mediated by resistance factor R-GN238.

Previously published work (Yamagishi *et al.*, 1969; Dale, 1971; Dale & Smith, 1971a) has shown that both enzymes have a pH optimum of 7.2–7.6 and an isoelectric point of 8.3.

There is now sufficient evidence to conclude that the R-1818-mediated β -lactamase and the R-GN238-mediated β -lactamase are not identical. This does not necessarily mean that they are unrelated, since Jack

& Richmond (1970) have found that some β -lactamases with marked differences in their enzymic properties are nevertheless indistinguishable with respect to their amino acid composition. Further, Ambler & Meadway (1969) have shown that similarities can even exist in the amino acid sequences of superficially distinct β -lactamases. However, the enzyme mediated by resistance factor R-1818 differs from other β -lactamases in so many respects that it would be surprising if it were closely related to any other β -lactamase yet described.

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