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Sequence of the OXA2 β-lactamase: comparison with other penicillin-reactive enzymes

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The nucleotide sequence of the unusual plasmid-mediated OXA2 β-lactamase is presented, and compared with other β-lactamases. The OXA2 enzyme has similar features at the presumed active site, but no other significant regions of homology with other penicillin-reactive enzymes. The active site homology may therefore represent convergent evolution of otherwise dissimilar genes.

β-Lactamase *Penicillin* *Antibiotic resistance* *Resistance plasmid* *DNA sequence*

1. INTRODUCTION

There are many different β-lactamases responsible for bacterial resistance to penicillins and other β-lactam antibiotics. Three classes of these enzymes have been defined on the basis of sequence homology [1]: class A enzymes, including those from *Staphylococcus aureus, Bacillus licheniformis, B. cereus* (type I) and the plasmid-mediated TEM β-lactamase show about 20% homology in their amino acid sequences [2]. The other classes of β-lactamases currently designated are class B, which contains the zinc-requiring enzyme from *B. cereus* (type 2) and class C, comprising the chromosomally determined 'cephalosporinases' from Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. Enzymes from different classes show little homology except in the region containing the active site. This relationship extends to the penicillin-binding proteins where the penicillin-binding site has features in common with the active site of the β-lactamases, but there is little homology in the remainder of the sequence [3,4].

The OXA2 type of β-lactamase is a plasmid-mediated enzyme which is found in a small proportion of ampicillin-resistant clinical isolates of Gram-negative bacteria. The original example is specified by the resistance plasmid R46, originally isolated from *Salmonella typhimurium* [5]. This enzyme possesses a number of features in which it is different from nearly all other known β-lactamases, notably the ability to hydrolyse oxacillin and other isoxazolyl penicillins, its dimeric structure and its interaction with anthraquinone dyes such as Cibacron blue [6–9]. Because of the unusual properties of this enzyme, we were interested in determining its sequence so as to ascertain its relationship to other β-lactamases and penicillin-reactive enzymes.

2. MATERIALS AND METHODS

E. coli HB101 [10] was used as the host for plasmid cloning and JM103 [11] for cloning with the M13 phage vectors mp7, mp8 and mp9 [12]. The plasmid vectors used were pACYC184 [13] and pED815, a derivative (kindly supplied by Dr N. Willetts) of pBR325 in which the ampicillin resistance (TEM β-lactamase) gene has been inactivated by a deletion around the *Pst*I site. The OXA2 gene was obtained from the plasmid R46 or from pKM101 (a deletion derivative of R46) [14]. Restriction maps of both of these plasmids have

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been published [15–17]. The plasmid pSU5 was constructed by inserting the appropriate *Bgl*II fragment of R46 into the *Bam*HI site of pED815; this plasmid was found to have suffered a deletion which did not appear to affect the β-lactamase gene. The *Bam*HI-*Hin*dIII fragment of pSU5 was then cloned by ligating with *Bam*HI-*Hin*dIII digested pACYC184 to yield the plasmid pSU8. Simplified maps of these plasmids are shown in fig.1.

Restriction endonucleases and other enzymes were obtained from commercial sources, and [^{32}P]dATP and [^{35}S]dATP for sequencing were from Amersham. Standard procedures for isolating and manipulating DNA were essentially as described by Maniatis et al. [18]. DNA sequencing was performed using the chain-terminating (dideoxy) procedure [19]. For sequencing, templates were prepared from recombinant M13 phages as described by Messing and Vieira [12]. The primers used were a 17 bp primer (Celltech) and a 26 bp primer (BRL).

Sequencing data were collected and analysed using the computer programs developed by Staden [20]; a version of these programs adapted to run on a Prime computer was supplied by K. Indge. Sequence comparisons and other routines were carried out using programs supplied by J. Pustell [21,22]; we are grateful to Dr T.N. Bryant for the implementation of both sets of programs.

3. RESULTS AND DISCUSSION

The sequencing strategy, summarised in fig.2, involved a combination of specific and random clones. Specific clones were obtained using the

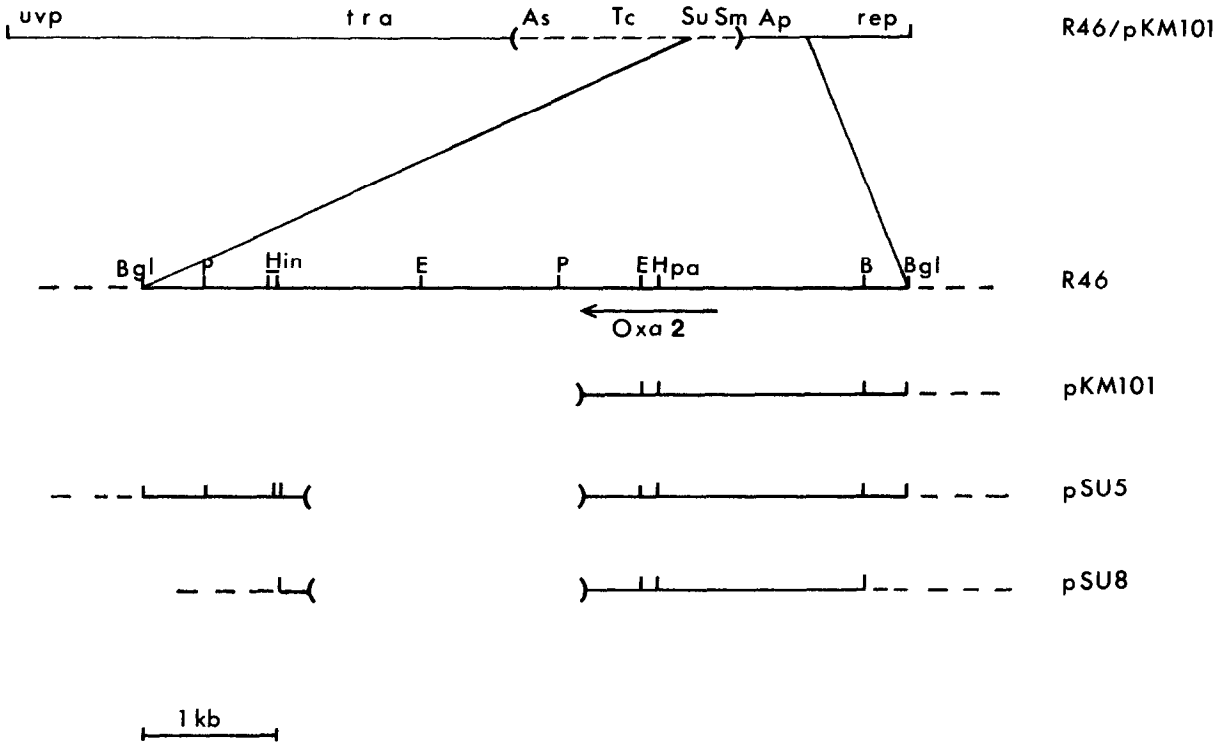

Fig.1. Structure of R46 and derived plasmids. The top line represents the structure of R46 (51.7 kb), linearised at the *Sma*I site adjacent to the replication region of the plasmid. The broken line indicates the portion deleted in pKM101. The region containing the OXA2 β-lactamase gene is shown expanded in the lower part of the figure, together with the corresponding regions of pKM101, pSU5 and pSU8. uvp, ultraviolet protection; tra, conjugal transfer functions; As, Tc, Su, Sm, Ap, resistance to arsenate, tetracycline, sulphonamides and ampicillin, respectively; rep, replication. Restriction enzyme sites indicated: Bgl, *Bgl*II; P, *Pst*I; Hin, *Hin*dIII; E, *Eco*RI; Hpa, *Hpa*I; B, *Bam*HI.

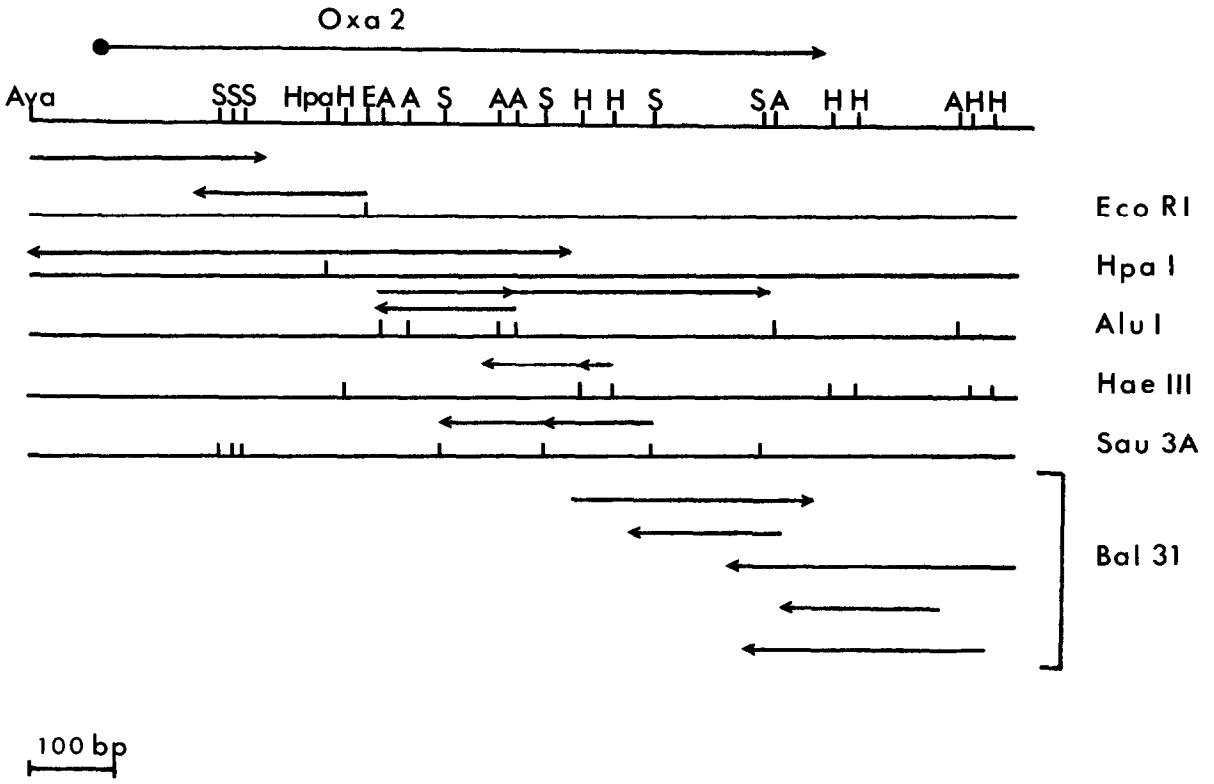

Fig.2. Detailed restriction map of the OXA2 β-lactamase gene and sequencing strategy. The location of cleavage sites for *Aval*, *Sau*3A (S), *Hpa*I, *Hae*III (H), *Eco*RI (E), *Alu*I (A), was deduced from the sequence. The lower part of the diagram shows the clones from which the sequence was determined; in many cases a single arrow represents several clones covering the same sequence. The entire sequence has been read from both strands, and with the exception of some of the sequence beyond the 3'-end of the gene, from independently isolated clones.

*Eco*RI, *Hpa*I and *Aval* sites. Random *Sau*3A fragments were generated from an isolated *Eco*RI-*Pst* fragment of pSU5 and random *Hae*III and *Alu*I fragments were obtained from the *Bam*HI-*Hin*dIII fragment of pSU5. Further clones were generated using *Bal*31 digests of the *Eco*RI-*Pst*I fragment. A sequence of about 1100 bases was obtained (fig.3) which shows a single open reading frame containing the known N-terminal amino acid sequence of the mature protein [23]. Initiation of translation at the first ATG codon upstream from this point would produce a signal peptide of 21 amino acids, predominantly hydrophobic. Seven bases separate this ATG from the sequence TAAGGA which is complementary to the 3'-end of 16 S rRNA and a putative ribosome binding site. The M_r of the mature enzyme is predicted as 29570, compared to the published values (by SDS gel electrophoresis) of 28000 [8] and 32170 [23] and the value of 44600 obtained by Sephadex gel filtration [6]. The amino acid composition of the protein predicted from the DNA sequence (table 1) agrees well with published results [6,23], thus confirming the DNA sequence data.

A comparison of this DNA sequence with that of the TEM β-lactamase [24] shows no significant homology (not shown). However, on comparing the amino acid sequences, 3 regions of homology were apparent (fig.4). One of these corresponds to the putative cleavage site of the signal peptide, although it is noticeable that this comparison would predict an additional histidine-alanine sequence at the N-terminal end of the mature OXA2 protein. The N-terminus was indeed originally identified as histidine [25] and the data reported here may account for this anomaly as well as for

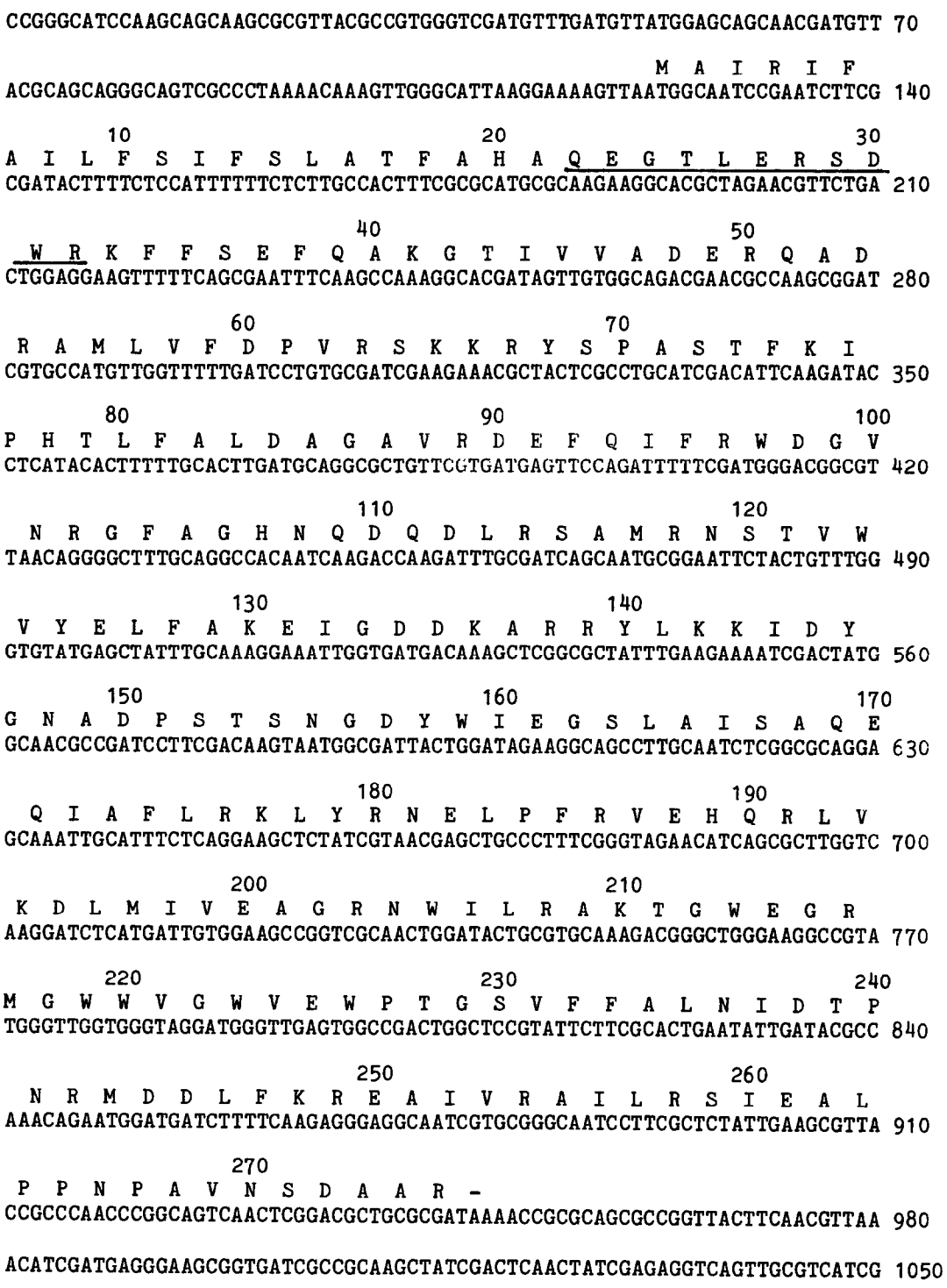

Fig.3. Sequence of the OXA2 β-lactamase gene. The amino acid sequence is numbered from the ATG initiation codon. The numbers in the column on the right refer to the nucleotide sequence, numbered from the *Aval* site. The underlined sequence shows the previously determined N-terminal amino acid sequence of the mature protein [23].

Table 1

Amino acid composition of OXA2 β-lactamase

Amino acid residue	Deduced from sequence		Recalculated from published data	
			[23]	[6]
Lysine	13		15.4	17.2
Histidine	4		3.6	3.5
Arginine	25		21.1	25.3
Tryptophan	10		5.6	11.7
Cysteine	0		0.8	–
Aspartate	19	30	28.9	27.9
Asparagine	11			
Threonine	9		11.3	8.8
Serine	14		14.2	13.9
Glutamate	16	25	27.6	25.4
Glutamine	9			
Proline	10		8.8	1.8
Glycine	16		18.5	16.9
Alanine	26		25.2	24.2
Valine	16		17.3	17.3
Methionine	5		5.4	4.7
Isoleucine	14		13.8	15.0
Leucine	18		20.0	20.4
Tyrosine	6		6.4	5.8
Phenylalanine	15		12.2	16.3

The mature OXA2 β-lactamase, starting from the histidine residue at position 20, contains 256 amino acid residues. The previously published values have been recalculated on this basis; –, not determined

the low yields of derivatives obtained in the N-terminal amino acid sequencing [23]. The second region of homology corresponds to the active site

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                    10        20        30        40
                    *         *         *         *
OXA2      MAIRIFAILFSIFSLATFAHAQEGTLERSDWRKFFSEFQA
TEM       hfrvalipfFaaFcLpvFAHpetlvkvkdaedqlgarvgy
                  *         *         *         *
                  10        20        30        40

          50        60        70        80        90        100
          *         *         *         *         *         *
OXA2      RQADRAMLVFDPVRSKKRYSPASTFKIPHTLFALDAGAVRDEFQIFRWDGV
TEM       eldlnegkileefRpeeRfpmmSTFKvllegavLsrvdagqEqlgrrihys
             *         *         *         *         *
             50        60        70        80        90

        150       160       170       180       190       200
        *         *         *         *         *         *
OXA2    DPSTSNGDYWIEGSLAISAQEQIAFLRKLYRNELPFRVEHQRLVKDLMIVE
TEM     wepelNeaipnderdttmpaamattLRKLltgELltlasrQqLidwmeadk
           *         *         *         *         *
           170       180       190       200       210
```

Fig.4. Comparison of selected regions of OXA2 and TEM β-lactamases.

region of the TEM enzyme, and indeed this is a feature which is conserved not only in class A β-lactamases but also to some extent in class C enzymes and in penicillin-binding proteins and other penicillin-sensitive enzymes [4,26]. The significance of the third apparent region of homology, centred on amino acid 190 of the TEM enzyme, is less certain; this does not appear to be a highly conserved region in other β-lactamases, and its appearance here may be merely fortuitous.

When the sequences of the 4 class A β-lactamases are lined up, they match completely in around 20% of their amino acids, with only a few padding characters needed [1,2]. Comparison of the OXA2 β-lactamase with these 4 sequences shows that the only region which matches (to an extent greater than expected by chance) is that adjacent to the active site region. Furthermore, it is not possible to increase the matching to any significant extent by inserting padding characters into either the OXA2 or the class A sequences. In addition, there is no obvious match between OXA2 and the published sequences of class C β-lactamases or with the penicillin-binding proteins [26–28]. It therefore seems that the OXA2 β-lactamase is not closely related to any of these enzymes; the homology in the active site region may be a consequence of convergent evolution.

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