THE DIMERIC NATURE OF AN R-FACTOR MEDIATED β-LACTAMASE

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ABSTRACT The molecular weight of the β-lactamase mediated by R46 as determined by SDS-gel electrophoresis is about half that of the native enzyme, indicating the presence of subunits. Similar evidence for subunits was found with the β-lactamase mediated by R55. *In vivo* hybridization occurred between the R46 and R55 β-lactamases.

INTRODUCTION

Ampicillin resistance mediated by transferable resistance factors (R-factors) in Gram-negative bacteria is associated with the production of β-lactamase (penicillinase; penicillin/cephalosporin β-lactam amidohydrolase, E.C.3.5.2.6.). R46 β-lactamase (molecular weight 44,600) is the prototype of a group of enzymes unusual both in their ability to hydrolyse oxacillin and in their high molecular weight, which is approximately twice that of other R-factor mediated β-lactamases [1]. Another member of this group, the β-lactamase of R55, was shown by us [2] to be distinguishable from the R46 enzyme by starch gel electrophoresis, and by its molecular weight of 41,000.

This paper presents evidence that the R46 and R55 β-lactamases both consist of two subunits. This is believed to be the first demonstration of subunit structure in any β-lactamase.

MATERIALS AND METHODS

Bacterial strains and R-factors. The strains of *Escherichia coli* K12 used were: 58.161/*sp met*$^-$; J53 *pro*$^-$, *met*$^-$; and J62 *lac*$^-$ *trp*$^-$, *his*$^-$, *pro*$^-$. The R-factors used were R46 (conferring

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resistance to ampicillin, streptomycin, tetracycline and sulphonamides; compatibility group N) and R55 (conferring resistance to ampicillin, chloramphenicol, sulphonamides and gentamicin/

kanamycin; compatability group C)[2]. R-factor transfer was performed as described by Smith [3].

β-lactamase activity was measured by the hydroxylamine method [4]. One unit of enzyme activity is that amount of enzyme which will catalyze the hydrolysis of 1μmol of benzylpenicillin per minute at 30°C and pH 7.4. Protein concentration was measured as described by Waddell [5].

The standard protein markers used for SDS acrylamide gel electrophoresis were: chymotrypsinogen (bovine pancreas), pepsin (hog stomach), alcohol dehydrogenase (yeast), ovalbumin (chicken egg), myoglobin (horse) - all obtained from Koch-Light Laboratories; trypsin (bovine pancreas), from B.D.H., and bovine serum albumin from Armour Pharmaceuticals.

Starch gel electrophoresis in borate buffer, pH 8.5, was performed as described previously [2], except that the buffer concentration was reduced to 0.02M in order to increase resolution. Crude enzyme extracts and other methods were as published [2,4].

RESULTS AND DISCUSSION

Purification of the enzymes. The R46 β-lactamase was purified by the method previously described [4]. The final preparation had a specific activity of 160 units per mg protein, which agreed well with the published value of 143 units per mg protein [4]. The β-lactamase mediated by R55 was purified by the same method, giving an enzyme preparation with a specific activity of 35 units/mg protein. The specific activity of pure R55 β-lactamase is therefore approximately one quarter of that of the R46 enzyme. This ratio is similar to that observed in crude extracts of the two enzymes [2]. From these figures and previous results [2] the number of enzyme molecules per bacterial cell can be estimated to be between two and three thousand for both the R46 and R55 β-lactamases.

Because of the basic nature of the R46 β-lactamase it is not possible to test the purity of the preparation by acrylamide gel electrophoresis under the normal anionic conditions [4]. The R46 and R55 enzymes were therefore run on acrylamide gels at pH 4.3 as described by Reisfeld, Lewis and Williams [6]. Four bands were obtained with either enzyme. These bands are probably not due to impurities in the preparations, since only one band is obtained on SDS gel electrophoresis (see below). Furthermore, in pH 4.3 electrophoresis, the same pattern of bands is seen with different purified preparations of R46 and R55 β-lactamases, and also with the similar β-lactamases of R 0x176 and R 0x179 (unpublished). These bands are thought to result from modification of the enzyme, and by partial dissociation into subunits, both induced by the low pH. Such presumed pH-induced modification of the R46 β-lactamase could be related to the storage-induced modification of this enzyme noted previously [2,4].

SDS-acrylamide gel electrophoresis. The purified R46 and R55 β-lactamases were subjected to electrophoresis in polyacrylamide gels in the presence of 0.1% sodium dodecyl sulphate (SDS) with markers of known molecular weight, as described by Weber and Osborn [7]. In some runs, the markers and enzyme samples were treated with boiling 0.01M phosphate buffer pH 7 containing 1% SDS and 1% 2-mercaptoethanol for two minutes in order to remove any possible proteolytic activity [8]. Similar results were obtained using samples prepared by either method. The R46 and R55 β-lactamases both migrated as a single band corresponding to molecular weights

of 28,400 and 29,000, respectively. These values are considerably lower than the values of 44,600 and 41,200, respectively, obtained by Sephadex gel filtration of the native enzymes [2]. We conclude from these results that both enzymes are dimers in the native state. If this is so, and if the monomer molecular weights are accurate, it implies that the molecular weights obtained by gel filtration are lower than the true molecular weight of the dimers. This could be due, either to the dimers being non-globular, or to the existence of a rapid monomer-dimer equilibrium with different dissociation constants for the two enzymes.

Formation of hybrid enzymes. As previously reported [2] the R46 and R55 β-lactamases can be distinguished by starch-gel electrophoresis (see also Fig. 1 a and b). If the subunit hypothesis is

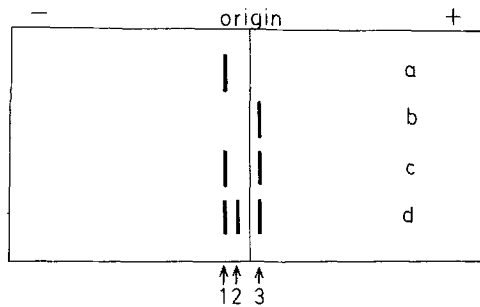

Figure 1: Diagrammatic representation of starch-gel electrophoresis of β-lactamases. The enzyme bands were visualised by means of a benzylpenicillin-iodine spray [2]. In practice, bands 1 and 2 develop more rapidly and have spread before band 3 becomes visible. This is due to the much lower level of activity of the R55 β-lactamase.

a) Result obtained with E.coli J62 (R46) or with a transcipient E.coli 58.161/sp (R46).
b) Result obtained with E.coli J62 (R55) or with a transcipient E.coli 58.161/sp (R55).
c) Result obtained with a mixture of cell extracts obtained from E.coli J62 (R46) and E.coli J62 (R55).
d) Result obtained with E.coli J53 (R55/R46) = strain JD 77.

correct, it might be possible to obtain a hybrid form of enzyme containing one R55 and one R46 subunit. No such hybrid form was seen on electrophoresis of a mixture of the two enzymes (Fig. 1c), nor did dialysis of the mixture against the buffer used for Sephadex molecular weight determinations [4] yield a hybrid form. This seems to rule out the hypothesis than an equilibrium exists between monomers and dimers during Sephadex gel filtration.

We have so far been unable to recover enzyme activity after dissociative treatment by SDS, guanidinium chloride or urea. Hence this approach could not be used in the construction of an artifical hybrid enzyme.

However, since the two R-factors are of different compatability groups and carry different resistance markers, it is possible to construct a strain carrying both plasmids, which would possibly produce a hybrid enzyme. A similar approach was used by Shaw _et al_ [9] in the study of R-factor specified chlorampenicol acetyltransferase.

R46 was therefore transferred from _E.coli_ J62 to _E.coli_ J53 (R55), with selection for transfer of tetracycline resistance. The colonies obtained were found to possess all the resistance markers of R55 and R46. One was chosen for further study. This strain, JD77, when used as a donor, was found to transfer each plasmid independently to _E.coli_ strain 58.161/_sp_. This indicates that R-factor recombination had not taken place in the doubly R^+ host.

When a crude cell extract obtained from strain JD77 was tested by starch gel electrophoresis, bands corresponding to the R46 enzyme (mobility towatds cathode +0.7 cm/hr) and the R55 enzyme (-0.2 cm/hr)

were observed. In addition, an extra band with a mobility of +0.25 cm/hr was seen (Fig. 1d). This may be attributed to the formation *in vivo* of a bybrid enzyme.

The transcipient *E.coli* 58.161/*sp* selected for transfer of the R46 resistance markers from strain JD77 was found to produce only the R46 β-lactamase (Fig. 1a). Similarly, the *E.coli* 58.161/*sp* (R55) transcipient produced only the R55 β-lactamase (Fig. 1b). This confirms that the hybrid enzyme was not formed by recombination within the β-lactamase genes.

Therefore these separate lines of evidence support the hypothesis that both the R46 and R55 β-lactamases are dimeric in their native state. It will be interesting to see what relationship, if any, there is between these subunits and the other oxacillin-hydrolysing β-lactamases, such as that of R_{GN238}, which have molecular weights of 24,000 in the native state.

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