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A comparison of the exo-penicillinases mediated by a chromosomal gene in a strain of *Staphylococcus aureus* PS 80 and by a plasmid gene in *Staphylococcus aureus* 8325

By M. H. RICHMOND

Department of Bacteriology, University of Bristol, University Walk, Bristol BS8 1TH

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There has been much discussion about the evolutionary source of plasmid-borne genes and their relationship to genes specifying a similar biochemical character carried on the bacterial chromosome (see, for example, two recent papers: Watanabe, 1971; Dale & Smith, 1971). One possibility is that such genes originated on the chromosome and were mobilized from that location by recombination with a plasmid to reach the extrachromosomal state (Campbell, 1962; Broda, Beckwith & Scaife, 1964). Although such excision processes do occur in laboratory strains, the difficulties of observing the process in the external environment are considerable and all the experiments that can be devised have an element of artificiality about them. If, however, the β-lactamase gene in a wild-type strain where the enzyme is chromosomally mediated gave a product indistinguishable from that found in wild-type strains where the gene is plasmid-borne, this would be evidence of the very close evolutionary relationship of the two genes concerned. Whether the primordial form of the gene was chromosomal or extrachromosomal cannot, of course, be as easily decided.

Ideally the identity of plasmid- and chromosomally-mediated exo-penicillinases in *Staphylococcus aureus* should be proved by detailed amino acid sequence studies, but this is a long and tedious process even though in this case the complete sequence of the plasmid-mediated enzyme is already known (Ambler & Meadway, 1969). Another sensitive method of comparison is to compare the chromatographic behaviour of the two enzymes when run as a mixture under conditions where any difference between the components is magnified to the greatest possible extent. In general, the extent of the evolutionary difference between two proteins is thought to be proportional to the number of amino acid transpositions in the primary sequence of the proteins, and there is no evidence to suggest that non-polar amino acids are any less involved in this process (see, for example, Goodman *et al.* 1971). Accordingly, even a limited amount of evolutionary difference between these two types of staphylococcal exo-penicillinase is likely to involve a small change in net charge on the protein and this should be reflected in the chromatographic behaviour of the molecule even though there may be no effect on the substrate profile (cf. the properties of the A- and C-type penicillinases from *S. aureus*: Richmond, 1965). Electrophoretic methods of examining such differences are unsatisfactory since it is often difficult to get clear patterns with staphylococcal penicillinase in starch or acrylamide gels.

To examine chromatographic behaviour, the $^{3}H/^{14}C$ ratio of each chromatographic fraction was determined when a mixture of purified ^{3}H-labelled exo-penicillinase from a plasmid carrying strain and ^{14}C-labelled enzyme from a strain with a chromosomal gene was chromatographed on a CM-cellulose column. The $^{3}H/^{14}C$ ratio obtained was very

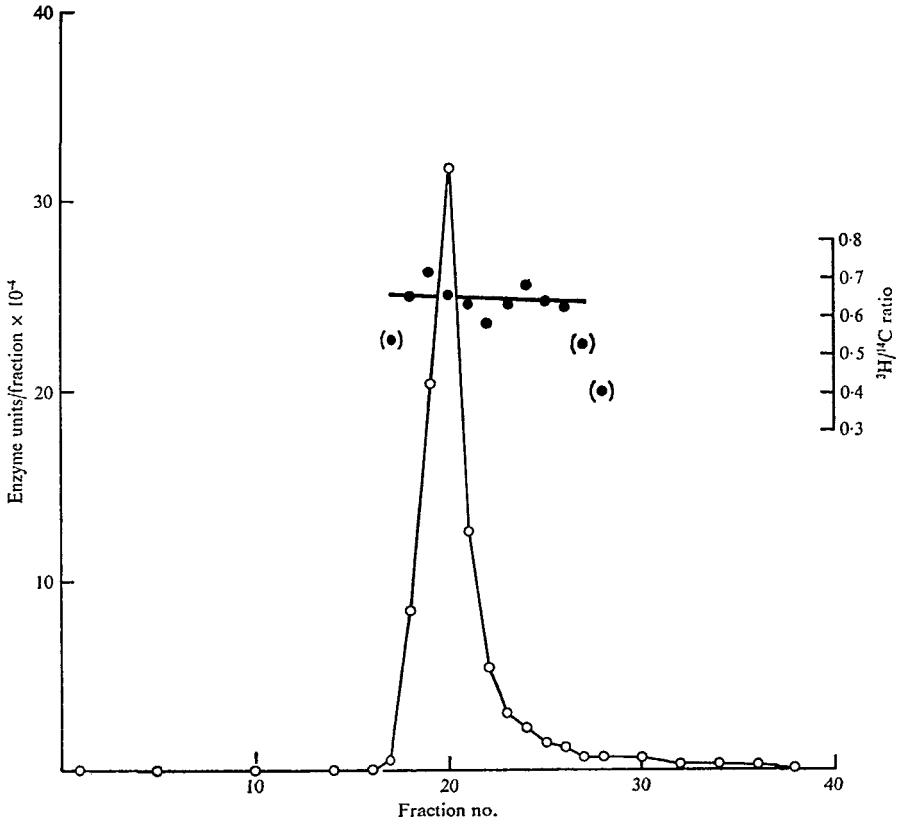

Fig. 1. Chromatography on CM-cellulose of a mixture of ^{3}H-labelled exo-penicillinase from *S. aureus* strain 8325($\alpha.i^{-}p^{+}$A) and ^{14}C-labelled exo-penicillinase from *S. aureus* Ps 80.$i^{-}p^{+}$(N). A mixture of $1{\cdot}7 \times 10^{5}$ units of 'chromosomal' enzyme containing $4{\cdot}5 \times 10^{4}$ c.p.m. and 3×10^{4} units of 'plasmid' enzyme containing $2{\cdot}7 \times 10^{4}$ c.p.m. Enzyme activity, ○; ^{3}H/^{14}C ratio, ●. Bracketed values on the graph are those that are too low for the quotient to be significant.

similar in each fraction from the column and this is evidence for a very close similarity of the two proteins, particularly since any difference in the chromatographic properties of the two components should give an increased ratio on one side of the peak and a reduced ratio on the other.

The two strains used in these experiments were *Staphylococcus aureus* carrying an α-plasmid specifying constitutive Type A exo-penicillinase synthesis (strain 8325 ($\alpha.i^{-}p_{A}^{+}$); see Richmond (1965, 1968) for nomenclature) and a variant of *Staphylococcus aureus* strain PS 80 in which the penicillinase gene is chromosomal (Asheshov, 1966). The variant was PS 80.$i^{-}p^{+}$(N) – a strain that lacked the plasmid specifying resistance to Cd^{2+} and to Hg^{2+} ions normally found in strain PS 80 (Asheshov, 1969). Strain PS 80.$i^{-}p^{+}$(N) was grown (1 l. culture) in 0·3 CY medium (Novick, 1962) containing 0·4 % (w/v) glucose and ^{14}C-labelled *Chlorella* hydrolysate (25 μCi: 37 mCi/milliatom C) while strain 8325 ($\alpha.i^{-}p_{A}^{+}$) was grown in similar medium containing ^{3}H-L-leucine + ^{3}H-L-valine + ^{3}H-L-tyrosine (0·5 mCi each: L-leucine, 0·5 C/m-mole; L-valine, 7 Ci/m-mole and L-tyrosine, 20 Ci/m-mole). Extracellular penicillinase was purified from both strains by the method published previously (Richmond, 1963) and the purified enzymes filtered

separately through columns of G 75 Sephadex equilibrated against KH_2PO_4/Na_2HPO_4 buffer, pH 6·5, to remove any low molecular weight radioactive contaminants.

A mixture of the two enzymes was then made ($1{\cdot}7 \times 10^5$ units of 'chromosomal' enzyme containing $4{\cdot}5 \times 10^4$ c.p.m. ^{14}C plus 3×10^4 units of 'plasmid' enzyme containing $2{\cdot}7 \times 10^4$ c.p.m. ^{3}H) and the mixture (2·0 ml) chromatographed on a CM-cellulose column prepared and run as described by Richmond (1963). Samples (1·7 ml) were collected on an Ultrorac fraction collector (LKB Instruments, Sweden). The β-lactamase content of each fraction was determined iodometrically by the method of Perret (1954) as modified by Novick (1962), and the $^{3}H/^{14}C$ ratio determined by scintillation counting. Fig. 1 shows the distribution of β-lactamase and the $^{3}H/^{14}C$ ratio in the column eluate. A single β-lactamase peak was obtained in which 93 % of the enzyme activity was present in 9 fractions (53 % recovery of the loaded material) and the $^{3}H/^{14}C$ ratio was approximately constant across the peak: certainly there was no evidence for a higher-than-average value on one side and a lower-than-average value on the other. One may conclude therefore that the two exo-penicillinases examined by this method were extremely similar in their properties; and this is likely to reflect the evolutionary origin of the two genes that specify them. Certainly the structure of the two genes, as inferred from the properties of their products, must be much more similar than the genes that specify the Type A and Type C exo-penicillinases, both of which are plasmid-mediated, but which contain a number of primary sequence differences involving charged amino acids (Richmond, 1965).

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