Progress on penicillinase

from David R. Thatcher

A workshop was held at the University of Newcastle upon Tyne from April 16 to 18 to discuss the biochemistry of bacterial penicillinases.

PENICILLINASES, or as they are now known, β-lactamases, are enzymes which specifically deactivate penicillin by hydrolysis of the β-lactam ring. Antibiotic resistance in a number of pathogenic bacterial strains has been conclusively linked with the production of these enzymes. In recent years transmissible plasmids or R factors carrying β-lactamase genes have been responsible for the rapid spread of resistance among organisms previously sensitive to penicillin. Penicillinases are therefore of immense medical importance and a detailed knowledge of their structures and mechanisms will be crucial in the development of new approaches to penicillin chemotherapy.

The present uses of β-lactamases in pharmaceutical research were outlined by G. W. Ross (Glaxo, Greenford). He pointed out that as new prospective antibiotics are synthesised it is essential that they be exposed to as wide a spectrum of naturally occurring β-lactamases as possible. During the past few years the interest of the drug companies has shifted from the gram-positive producers like *Bacillus cereus*, to gram-negative producers like *Pseudomonas aeruginosa* and *Escherichia coli*, as the greater medical significance of the latter group and their R factors was realised. β-Lactamases from a large number of gram-negative sources have been characterised at Glaxo, using immunological techniques and a new isoelectric focusing method. Several of these enzymes were purified to homogeneity using an interesting single-step QAE-Sephadex column chromatography method. J. W. Dale (University of London) reviewed the range of penicillinase types determined by R factors, including a fascinating class of dimeric β-lactamases having a high specificity for oxacillin.

It is becoming increasingly apparent, however, that any fundamental advances in antibiotic design will be based on our physico-chemical understanding of the interaction of the drug with the target enzyme (in this case the cell wall transpeptidase) and any specific resistance mechanisms (in this case, penicillinase). Structural studies at the moment are still governed by the availability of large quantities of pure enzyme and consequently most of our information is confined to the β-lactamases of gram-positive super-producing strains of little medical importance.

Complete amino acid sequence data are available on the enzymes from *Staphylococcus aureus* and *Bacillus licheniformis* with partial data on the sequence of the β-lactamase I of *Bacillus cereus* (80%) and preliminary data on the *E. coli* TEM enzyme. R. P. Ambler (University of Edinburgh) explained how the three gram-positive sequences known show a high degree of similarity to each other whereas the fragmentary data available on the *E. coli* TEM enzyme show little, if any, evidence for homology.

X-ray crystallographic analysis of two enzymes, the *E. coli* TEM enzyme (J. R. Knox, University of Connecticut) and the *S. aureus* penicillinase (D. W. Green, University of Edinburgh) has commenced and L. Sawyer and J. Moult (University of Edinburgh) presented a report of progress made up to now. It is hoped that low resolution structures will soon be completed for both enzymes.

The conformation of the *S. aureus* penicillinase in solution has been examined by difference spectroscopy, optical rotatory dispersion and circular dichroism (R. H. Pain, University of Newcastle upon Tyne). Gradually unfolding the protein in increasing concentrations of guanidine hydrochloride revealed a partially unfolded state(s) which although retaining all α-helical structure, was enzymologically inactive and appeared, on the basis of optical absorption and flow properties, to be expanded, with the 13 tyrosine residues fully exposed to solvent.

By analysing the equilibrium constants of the transitions between the native, partially unfolded and fully unfolded states, Pain and co-workers have been able to calculate the free energies of the two denatured states, with respect to the native state. These values are much lower than, for example, those found in the denaturation of ribonuclease or lysozyme. *S. aureus* penicillinase could in fact be described as a 'floppy' molecule and Pain has calculated that in solution up to 3% of the enzyme molecules are in the partially unfolded state(s). Conformational flexibility is becoming a characteristic property of β-lactamases or, more specifically, conformational changes provide the best explanation of a number of unusual pencillinase properties.

The search for residues involved in the catalytic site of β-lactamases continues without marked success. S. G. Waley (University of Oxford) presented k_{CAT}/K_{M} against *p*H plots for the hydrolysis of benzylpenicillin by the β-lactamase of *B. cereus*. The usual bell-shaped plots appeared and these results were explained on the basis of the ionisation of groups in the catalytic centre (pK_as of 5.5 and 8.6) affecting substrate binding. The group with a pK_a of 5.5 could either be a carboxyl or an imidazole side chain, Waley tentatively favouring the former on the basis of inactivation studies using Woodward's reagent. Waley also described a number of elegant inhibition experiments using penilloates (decarboxylation products of penicilloic acid) and showed that these compounds were much better inhibitors than the natural penicilloic acid products.

Stopped-flow spectrophotometric data (S. Halford, University of Bristol) using the *S. aureus* enzyme and a chromogenic substrate showed that in this case the bell-shaped plot of k_{CAT} against *p*H could arise by the change of one rate-determining step to another with *p*H. Rapid reaction kinetics resolved the reaction into three phases, the formation of (ES), conversion of (ES) to (EP) and the decomposition of (EP) to the free enzyme and product. Below *p*H 6.0 the reaction rate is limited by the conversion of (ES) to (EP) and above *p*H 6.0 by the decomposition of (EP).

Using a *p*H indicator in the assay system, Halford was able to show that proton release accompanied the (ES) to (EP) transition; a result which favours a general acid-base catalytic mechanism rather than a hypothesis involving the formation of a covalent acyl intermediate. If the release of this proton is involved in the rate-limiting decomposition of (ES), a primary isotope effect should be observed when the reaction is performed in D_2O. As no such effect was observed the rate of conversion of (ES) to (EP) probably also involves a rate-limiting conformational change after proton release.

R. Virden (University of Newcastle upon Tyne) and coworkers have been studying the effect of quinacillin inhibition on *S. aureus* penicillinase. This penicillin analogue is a substrate, albeit a poor one, yet it progressively inactivates the enzyme and on subsequent dilution into benzylpenicillin solutions the inhibited enzyme only slowly regains normal enzymic activity.

Kinetic analysis of this reversible inhibition has been performed and was explained in terms of quinacillin binding preferentially to an inactive conformation of the enzyme. Denaturation during quinacillin hydrolysis yields a covalent enzyme–substrate complex. Chemical analysis of this complex has not yet shown whether or not quinacillin is bound at a single site.

High resolution structural data on a β-lactamase will be invaluable in the interpretation of many of the kinetic and chemical modification data. Apart from the potential benefits to medicine, it looks as if future research on penicillinases will make many valuable contributions to our understanding of enzyme function in general.

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