Microbiology

Proteins that bind the β-lactam antibiotics

from A.F.W. Coulson

A MEETING that is concerned more with the enzymes that form the targets for the β-lactam antibiotics than with the β-lactamases that destroy them, would not sensibly be called a 'β-lactamase workshop'. But the name was coined in 1975 for the first of the workshops; by the third*, held recently, the focus had changed.

The target enzymes can be identified in whole cells by their binding to ^{14}C-penicillin G. In *Escherichia coli* there are two classes of penicillin-binding protein (PBP). The low-molecular-weight proteins, PBP-4, -5 and -6, are not essential to the organism, are DD-carboxypeptidases and are believed to regulate the degree of cross-linking of the cell wall. The high-molecular-weight PBP-1a, -1b, -2 and -3 have already been shown to be essential to *E. coli*; real progress in understanding the behaviour of these membrane-bound proteins is now becoming possible through the cloning and sequencing of their genes.

The sequence of the PBP-3 gene, representing a protein of 588 amino acids, was reported a year ago by Y. Hirota (National Institute of Genetics, Mishima). PBP-3 displays both transpeptidase and transglycosylase activities, and the latter is not inhibited by penicillins. B. Spratt (University of Sussex) presented evidence that the transpeptidase activity is associated with the carboxy-terminal half of the molecule, whereas the amino-terminal half contains the transglycosylase activity.

J. Broome-Smith (University of Sussex) has sequenced the genes for PBP-1A, PBP-1B and PBP-5. 1A and 1B are highly homologous; otherwise the only extensive sequence similarities in this group occur in the region of the tetrapeptide -Ser-X1-X2-Lys-. Tetrapeptides of the same form also occur in low-molecular-weight DD-carboxypeptidases and in several examples of each of two types of β-lactamase. For β-lactamase it has been very thoroughly established that the serine residue is part of the active site, and is acylated by a β-lactam in the course of its hydrolysis. Other similarities in the sequences of these three groups of enzymes are also concentrated in the neighbourhood of the tetrapeptide sequence. It is clearly very tempting to suppose both that the serine residue is part of an active site with a similar role in the mechanisms of all the enzymes, and that all the genes are homologous, at least in parts.

A highlight of the meeting was the presentation of the first high-resolution X-ray crystal structure of one of the proteins that interact with penicillins: the 2.8Å structure of a *Streptomyces* DD-carboxypeptidase (J. Kelly, University of Connecticut). The enzyme contains two regions of secondary structure — a six-strand β-sheet and a group of five stacked α-helices. To add to the progress that has been made in the last four years in extending the sequence-based classification of β-lactamases, R. Ambler (University of Edinburgh) reported a partial sequence of the Zn-containing β-II enzyme from *Bacillus cereus*, still the only member of class B. And J.O. Lampen (Rutgers University) presented a partial sequence of its gene.

The most interesting mechanistic study was that described by R.F. Pratt (Weslyan University, Connecticut) who showed that acyclic esters of the form $Ar.CH_2.CO.NH.CH_2.CO_2R$ are hydrolysed by β-lactamases — the first non-β-lactam substrates to be discovered. Transpeptidation can occur from these substrates to suitable amines, which implies that acyclic amides must also be substrates (J.R. Knowles, Harvard University).

The induction of penicillinase in bacilli has unusual features which have made it a long-standing problem, a solution to which may be at hand now that clones of the entire penicillinase operons are available (Lampen). It seems clear that the main oddities in the induction arise because the inducer modifies the cell wall giving rise to a 'second messenger' within the cell; the genetic control itself may be entirely conventional. The nature of the modification to the cell wall remains mysterious, particularly since it can be brought about by a variety of chemically very different agents.

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Astronomy

Quadrantid meteors

from David W. Hughes

IT is none too easy to gather data on the Quadrantid meteoroid stream. January 3 or 4, depending on our phase in the leap-year cycle, is the only day of the year on which the Earth intersects the stream; the point on the celestial sphere from which the shooting stars appear to originate has a declination of + 48.5°, so Quadrantids are best seen from high latitudes in the Northern Hemisphere where winter makes visual and photographic conditions seldom satisfactory; and at least one year in four is hampered by moonlight. Moreover, the Quadrantid stream is very narrow, the time between half-maximum flux rates being about 12 hours, and if the maximum occurs when the radiant is low in the sky, the proportion of the flux that can be observed by any method is much reduced. This factor limits optimum observations of the shower to one year in every four.

Radar methods can overcome two of the problems, permitting the observation of meteors in daylight and through clouds. They, however, suffer from their own kind of 'bad weather', because radio propagation conditions in the ionosphere can vary considerably as a function of solar cycle. Another problem with radar is the identification of the shower meteors. This is usually accomplished simply by subtracting an assumed background rate from the total observed rate.

One goal of meteor stream investigations is to unravel the disintegration process of the parent comet and the subsequent evolution of the meteoroid stream. A knowledge of the particle number density across the stream and around the stream orbit, and the way both of these vary as a function of particle size, would be very useful in that context. Hence the value of the determination by B.A. McIntosh, of the Herzberg Institute of Astrophysics, Ottawa, Canada, and M. Šimek, of the Astronomical Institute, Ondřejov, Czechoslovakia, of the long-term average cross-section of the Quadrantid meteoroid stream by analysing radar data obtained over nearly a quarter of a century (*Bull. astr. Inst. Czechoslovakia 35*, 14; 1984).

The Ondřejov and Ottawa radars are both pulsed systems and are similar in operation. Typical parameters are pulse length 10 μs, peak power 25 kW, repetition frequency 500 Hz. The antenna is fixed at an elevation of 45° and meteors within a line-of-sight distance of 400 km are recorded. Meteors are sorted into three duration classes, equivalent to median magnitudes of + 6, + 1.6 and − 1.0.

The practice of adding one year's data to the next is common with Quadrantid researchers but McIntosh and Simek warn that it is not without problems. These become obvious when the combined data for the time interval 1958–81 are compared with the well-observed year of 1979. There are three basic numerical values which represent any flux curve: the time of maximum flux, the rate of rise and the rate of decay. The last two can be combined to give a stream width which is usually defined as the change of solar longitude that occurs during the time when the stream is more active than half its maximum activity. The

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authors noticed that the stream width from the combined data was twice that of 1979. The cause, they say, is the variation in the position of maximum, which can change by as much as 0.5° in solar longitude from year to year. So the annulus of Quadrantid meteoroids in the Solar System is not perfectly elliptical and deviations from this ellipse, at the descending node, can be as much as 7×10^5 km.

Contrary to the findings of many researchers, McIntosh and Šimek found that the maximum flux of bright meteors did not inevitably occur after the maximum flux of faint ones; in certain years the bright meteors preceded the faint ones. For all three meteor magnitudes the rate of rise of the flux to the maximum was slower than the subsequent fall. It was found, however, that the asymmetry increased as one considered brighter meteors. The theory of meteor stream production predicts that width should decrease as a function of meteor brightness. The authors found that the + 6.0 and − 1.0 magnitude meteors obeyed this rule but that the + 1.6 magnitude meteors had a wider flux curve than either of the others.

In conclusion, it is clear that the Quadrantid shower, currently both one of the most active showers and the narrowest, has many peculiarities. These can, in the main, be blamed on Jupiter. The ascending node of the Quadrantid orbit occurs very close to Jupiter's orbit. Also the mean period of the Quadrantids is approximately half Jupiter's orbital period. This causes the nodes of the Quadrantid orbit to regress by about 0.4° per century, leading to mass sorting in the shower (smaller meteoroids usually peaking earlier than larger ones) and introducing short-term resonance fluctuations which again have a size-dependent effect.

Jovian perturbation is very selective. The small sector of the stream that is in the vicinity of Jupiter every 11.86 years will be shifted by larger amounts than the rest of the stream. The velocities of the perturbed meteoroids will be changed slightly and they will redistribute themselves around the orbit, eventually forming a low-density tail preceding the main body of the stream. McIntosh and Šimek conclude that the degree of asymmetry so produced is to some extent a measure of the age of the stream.

This leads us to one of the main unsolved questions concerning meteor streams. How old are they? We know that the Quadrantids are old enough for the meteoroids to have spread out fairly evenly around the orbit and for the parent comet to have completely disappeared. But the stream is still young enough to retain the record of slimness. If only we knew its age our ideas about stream evolution could be placed on a much firmer footing. □

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Molecular biology

DNA gyrations in reverse

from James C. Wang

In the well-known double-helix structure of DNA, the two strands revolve around each other. The topological problem of separating the parental strands during replication, particularly when the double-stranded DNA is in the form of a ring and thus the complementary strands are two intertwined single-stranded rings, was recognized more than two decades ago[1,2]. The enzymes capable of solving such topological problems, DNA topoisomerases, are ubiquitous. They catalyse the transient breakage of the DNA backbone and the passage of DNA strands through the transient breaks[3-5] in two mechanistically distinct ways. The type I enzymes break and rejoin one strand at a time and catalyse the crossing of one strand through another; the type II enzymes break and rejoin a pair of strands in concert and catalyse the crossing of a double-stranded DNA segment through a double-stranded break. It is not surprising that both types of activities are found in an archaebacterium *Sulfolobus*, as reported by Kikuchi and Asai on page 677 of this issue of *Nature*; what is amazing, however, is that *Sulfolobus* also contains an entirely new subclass of type II topoisomerase[6].

Until now, two subclasses of type II topoisomerases had been found in eubacteria and eukaryotes. Bacterial gyrases, which fall into one subclass, are characterized by their ability to reduce the 'linking number' of a double-stranded DNA ring. The term 'linking number' is a topological quantity defined as the number of times one strand revolves around the other when the duplex ring is laid in a plane. If a DNA ring laid in a plane assumes the B-helical form, 10.5 base pairs make a complete helical turn and the linking number is the total number of base pairs divided by 10.5. When a duplex DNA ring is incubated with gyrase and ATP *in vitro*, the linking number is reduced by as much as 10 per cent below that of the most stable B-DNA structure. Gyrase achieves this energetically unfavourable reduction in linking number, termed 'negative supercoiling', by coupling DNA supercoiling to ATP hydrolysis.

The other subclass of type II topoisomerase includes those found in eukaryotes and phage T4 topoisomerase. These enzymes, though ATP-dependent, can only break and rejoin DNA to allow the passage of strands in such a way that the linking number of a duplex DNA ring 'relaxes' towards that of the most stable DNA structure, whatever its initial linking number; in this process, the chemical energy of ATP hydrolysis is not harvested by the DNA.

By contrast, the type II topoisomerase found by Kikuchi and Asai in *Sulfolobus* increases the linking number above that of the most stable DNA structure by coupling ATP hydrolysis to the positive supercoiling of DNA. 'Reverse gyrase', as Kikuchi and Asai call their enzyme, thus becomes the founder member of a third subclass of type II topoisomerase, and an unexpected one, because no positively supercoiled DNA has ever been isolated from cells in physiological conditions.

Since all three subclasses are ATP-dependent and catalyse the passage of one double-stranded DNA segment through another, their differences lie in the directionality of duplex cross-over. Strand passage catalysed by the gyrase and reverse gyrase is directional, though opposite in sign, whereas strand passage catalysed by the other subclass lacks directionality and the DNA relaxes into a thermodynamic valley.

The discovery of reverse gyrase raises a number of questions. First, is it a distinct enzyme rather than an altered form of a gyrase of the same organism that has somehow changed its direction of strand passage? Mechanistically, it is an intriguing question whether directionality can be altered by changing the quaternary structure of an enzyme or by modifying its subunits. The scanty information that is available comes from studies with *Escherichia coli* gyrase. In the absence of ATP, gyrase can relax negatively supercoiled but not positively supercoiled DNA. However, a complex containing the A subunit of gyrase and what is probably a proteolytic fragment of the B subunit of gyrase, can relax both negatively and positively supercoiled DNA[7,8]. Moreover, gyrase is stimulated to relax positively supercoiled DNA by the binding of the β,γ-imido-analogue of ATP[9]. Whereas such experiments show that it is possible to nullify the normal bias in the directionality of strand passage (relaxation of negatively supercoiled DNA) by the modification of a subunit of gyrase, it is more difficult to envision how strand passage could be switched from one direction to another by subunit modifications. The total lack of directionality in strand passage catalysed by phage T4 and eukaryotic type II topoisomerases is as intriguing as the directional strand passage catalysed by the other type II enzymes. In the case of the eukaryotic type II topoisomerases, the purified enzymes are homodimers. It is plausible that complexes between such enzymes and DNA have a twofold symmetry axis and that the symmetry abolishes any bias in the directionality of strand passage. The interesting question, then, is whether the binding of additional protein factors, or the

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