

Even if the individual plots are non-linear, if the nonlinearity is present equally in both of the curves, due to conformational changes that affect the reaction of both substrates equally, their difference may still be linear. The method given for determining the apparent differential Eyring parameters is thus reasonable if the data are linear, as ours are. However, the magnitudes of these parameters are likely to be sensitive to other physical variables, such as pH, buffer structure, cofactors, cosolvents, etc., and so they must be obtained and compared under carefully controlled conditions.

Nonetheless, I recognise that k_{cat}/K_m for an enzymatic reaction is not an intrinsic constant, but a complex mixture of intrinsic kinetic constants related to both binding and chemical processes, which may differ in their individual activation parameters, and it is also possible that the

rate-limiting step may change with temperature. If so, then a 'turn-around' in the effect of T on E, as reported by Parmar *et al.*², may well occur. Examples of this behavior in stereoselective nonenzymatic reactions have been documented by Scharf and co-workers³. However, given the limited number of temperature data points and the lack of statistics of the measurements in the work of Parmar *et al.*², it is premature to conclude that these results are an enzymatic example of this principle.

Finally, Duan and Chen state that no one else has published T_r for an enzyme reaction. This may be true at the present time, but I am aware of other groups that have recently performed similar measurements, and I am certain that other cases of T_r measurements for enzymes will be forthcoming in the near future. However, 'isoenantioselective temperatures' have been published for the

separation of enantiomeric compounds by gas chromatography on chiral stationary phases^{4,5}. These systems show a reversal of elution order with increasing temperature, and the data were analysed by the use of a thermodynamic equation analogous to the equation given in my article.

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Meeting report

Target practice – novel approaches to antimicrobial chemotherapy

Much of the development of biotechnology has benefited greatly from the historical preeminence of microbially produced products (and their derivatives) that possess antimicrobial activity, but it is now generally recognized that bacterial resistance to these molecules is widespread. Thus, many classes of organism, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci, currently give enormous cause for concern in clinical settings, especially following the demonstration of the transfer of vancomycin resistance from enterococci to staphylococci. One reason for the emergence of such resistance is that the number of primary pharmacological targets actually exploited by these molecules is exceptionally small, and, driven in part by the genomics revolution, it is now widely recognized that the next generations of antibiotics should be aimed at new and different ones. This was the theme of a meeting held recently at the Institut Pasteur*.

*New targets for new antibiotics, organized by Jozsef Aszodi and Patrice Courvalin, Institut Pasteur, Paris, France, 4–6 June 1997.

Of the clinically useful antibiotics, approximately 55% are β -lactams, with macrolides and fluoroquinolones each comprising about 15% of the market, yet of possible new chemical entities only oxazolidinones are currently known to be in even phase-I clinical trials (Jozsef Aszodi, Hoechst Marion Roussel Research Centre, Romainville, France). Much of the problem of how to make progress follows from our general ignorance of how bacteria work – for instance, the genome of *Escherichia coli* strain O157 is 25% larger than that of strain K12, so simple subtractive methods of gene expression will rarely give clean answers; in addition, recognizing a good target is only the very first step to obtaining a good pharmacological agent (Julian Davies, University of British Columbia, Vancouver, Canada).

By definition, a good antibiotic target is one that is necessary for the growth of the target cell under most or all conditions, including *in vivo*, is typically found in most common pathogens (to give a broad spectrum of activity) and yet is not present in the human host. The bacterial cell-division process provides a number

of such targets, including the ftsZ ring (Joe Lutkenhaus, University of Kansas, Kansas City, KS, USA) and Min proteins (Jean-Pierre Bouché, CNRS, Toulouse, France) as do a variety of steps in peptidoglycan biosynthesis (Jean van Heijenoort, Université Paris Sud, Orsay, France); 2D zymograms of peptidoglycan hydrolases coupled to MALDI-TOF mass spectrometry have identified new targets, including skp (Wolfgang Keck, Hoffman-La Roche, Basel, Switzerland).

According to one view, that of 'rational drug design', the availability of a 3D crystallographic structure can make the process of drug design very much easier. The structure of elongation factor EF-Tu, which is involved in protein synthesis, is now available. This protein is known to have 60–80% conserved homology among bacteria but only 30% homology to the equivalent eukaryotic EF-1a, and, of a number of structurally distinct natural products that bind and block its activity (kirromycin, pulvomycin, MDL62,879), none is toxic to eukaryotes. EF-Tu is extremely abundant in *E. coli* and, although the above molecules are unsuitable for reasons of pharmacokinetics, their antimicrobial activity suggests that EF-Tu could indeed be an excellent target (Khalid Islam, Hoechst Marion Roussel Research Centre, Romainville, France).

Much of the growth, development and response of bacteria to a changing environment is mediated by the σ factors. These control which promoters will be read by RNA polymerase and, while drugs capable to binding to the interface between such a protein and nucleic acids are likely to be generally cytotoxic, the interface between the σ factors and the core enzyme presents an attractive target (Abraham Sonensheim, Tufts University, Boston, MA, USA).

During protein synthesis, premature dissociation of peptidyl tRNAs can lead to their cytotoxic accumulation; they are normally recycled by a peptidyl-tRNA hydrolase, and substances that prevent its activity are a potential source of antibacterial compounds (Sylvain Blanquet, École Polytechnique, Palaiseau, France). Bacterial proteins are initially formed with an N-terminal N-formyl methionine and, while the methionine group is usually removed in both prokaryotes and eukaryotes, the removal of the formyl group by a specific deformylase is specific to bacteria (Didier Mazel, Institut Pasteur, Paris, France). The over-expression (and consequent specific inhibition) of this enzyme can be assayed by the ability to grow on N-formyl-methionyl leucine as sole source of these amino acids.

While the cell wall of bacteria has long been recognized (*a posteriori*) as a suitable antibiotic target because of its unique chemistry, it is evident that not only its construction but also the secretory supply of its building blocks can serve this purpose. Of the three known secretory pathways, a general secretory pathway (GSP) is common to both Gram-positive and Gram-negative bacteria (Tony Pugsley, Institut Pasteur, Paris, France), as are ATP-binding cassette-type transport systems (Cécile Wandersman, Institut Pasteur, Paris, France). As with other cell-surface targets, the GSP is especially attractive as it avoids potential permeability problems and those due to efflux pumps that must be negotiated by molecules whose targets are cytoplasmic; *secA* may be particularly suitable. The type-III secretory pathway is involved in injecting bacterial proteins into foreign hosts, and is essentially unique to invasive Gram-negative bacteria such as *Yersinia* spp.; such a system can, of course, represent an antibacterial target, but might also be subverted for the intracellular delivery of vaccines and other therapeutic

proteins (Guy Cornelis, Université Catholique de Louvain, Brussels, Belgium).

The challenge of genomics is not the acquisition of the sequences *per se*, as the entire human genome could be sequenced if every inhabitant of China sequenced just three bases (perhaps, for safety, on each strand) (Sydney Brenner, Institute of Molecular Sciences, La Jolla, CA, USA), but for the bioinformatics to keep up with the flood of genomic data to enable it to be exploited to the full. Even the few (<10) whole microbial genomes (including baker's yeast) in the public domain have caused a reevaluation of phylogeny on a grand scale, and indicate that the 'minimal gene complement' of bacteria under the most favourable conditions (and thus, at the most simplistic level, the possible number of possible antibiotic targets) is some 250–300 (Eugene Koonin, National Center for Biotechnology Information, Bethesda, MD, USA). Horizontal gene transfer on a large scale greatly complicates the rRNA-based 'tree of life', in which the Archaea had been seen as inclined towards the eukaryotic branch. Automated tools now allow the reconstruction of metabolic pathways in organisms as they are sequenced (Evgeni Sel'kov, European Bioinformatics Institute, Cambridge, UK). As genomics leads to functional genomics, and we move swiftly to the post-genomic era, the flood of data will increase even more. The big pharmaceutical houses are already exploiting oligonucleotide chip arrays and 2D electrophoretic analyses of the proteome to assess the expression of the ribonucleome under different conditions (Chris Gray, Hoffman-La Roche, Basel, Switzerland). The data emerging from the mycobacterial genome sequencing projects (and, to the great credit of those involved, being made available on the Internet as soon as they are checked) have already produced many surprises in *Mycobacterium tuberculosis*, such as a series of polyketide synthases (and, consequently, many new targets for drugs and vaccines) and the basis for the problematic multidrug resistance in this organism, seen in the array of β -lactamases, drug efflux pumps, etc. that its genome can encode. Indeed, in contrast to *Mycobacterium leprae*, which is apparently on the way to being eradicated, deaths from *M. tuberculosis* are greater than ever, and specific narrow-spectrum drugs

must fill the 20-year gap before new classes of vaccine might become widely available (Stewart Cole, Institut Pasteur, Paris, France).

The problem of MRSA is so acute that an economic (as well as moral and scientific) case can be made for the development of drugs specifically to bypass methicillin resistance in such strains. Although its basis is the production of a novel penicillin-binding protein PBP 2', a number of other factors essential for methicillin resistance (fem factors) are involved in the production of unique pentaglycyl groups in the polymeric staphylococcal cell wall, providing a clear and immediate target (Harald Labischinski, Bayer AG, Wuppertal, Germany). The sorting of surface proteins in such Gram-positive bacteria involves transpeptidases, which could also provide good targets (Olaf Schneewind, UCLA School of Medicine, Los Angeles, CA, USA).

The basis of vancomycin resistance in enterococci is now fairly well understood at the molecular level, and mainly involves the synthesis of D-alanine-D-lactate linkages instead of the D-alanine-D-alanine intermediates of peptidoglycan biosynthesis to which glycopeptides such as vancomycin normally bind (Peter Reynolds, Cambridge University, UK); a single phe \leftrightarrow tyr change is sufficient reversibly to effect resistance.

Multidrug resistance via efflux pumps is a well-recognized problem in both prokaryotes and eukaryotes (where the P-glycoprotein can mediate the resistance of certain tumours to cytotoxic drugs), and the inclusion of inhibitors of such pumps in antimicrobial cocktails could clearly play an important role in overcoming resistance (Olga Lomovskaya, Microcide Pharmaceuticals, Mountain View, CA, USA). However, it is necessary to inhibit all such pumps simultaneously, and their lack of substrate specificity evidently causes difficulties in finding a suitable inhibitor that would be active at low concentrations.

As with human warfare, an important class of target involves the systems used by the 'enemy' to sense what is going on in their environment and to communicate this between themselves. In sensing, many of the environmental changes are detected and mediated via more-or-less-highly conserved '2-component' systems involving histidine kinases, which have no counterparts

in animals (Tom Silhavy, Princeton University, Princeton, USA); as *E. coli* has some 28 of these, resistance is unlikely to prove a problem. In communication between Gram-negative bacteria, an important general 'language' involves N-acyl homoserine lactones (Gordon Stewart, Nottingham University, UK); because these often induce their own synthesis, the environmental levels reflect the bacterial concentration, and can be used by the organisms (in 'quorum sensing') to determine when this is sufficient to make the expression of exoenzymes and other virulence factors profitable. Inhibiting their synthesis or activity could therefore block virulence. In fact, one such molecule (OdDHL) in *Pseudomonas aeruginosa*, an opportunistic pathogen, is itself a virulence factor, as it can inhibit the inflammatory response in which lipopolysaccharide (LPS) induces the formation of TNF- α in lymphocytes. Antimetabolites from red algae are already known.

Thus, given that bactericidal antibiotics induce autolysis, which

can lead to septic shock, it is reasonable to conclude that blocking the expression of virulence genes alone might be sufficient for effective antibiosis *in vivo*, and negative selection for multiple virulence genes *in vivo* is possible via signature-tagged mutagenesis (David Holden, Royal Postgraduate Medical School, London, UK); gene knockouts at loci identified in this fashion could decrease the dose of *Salmonella typhimurium* required for lethality by five orders of magnitude!

Of course there is much continuing interest in the exploitation of microbial diversity, and, indeed, of combinatorial biology, for the synthesis of novel antimicrobials. Certain polyketide synthases are synthesized as huge polypeptides that fold to form the modules of a helical, dimeric, multienzyme complex that adds groups to the growing polyketide *seriatim* (Jim Staunton, University of Cambridge, UK); mixing and matching modules (or, for that matter, mutating them or changing their order) could provide a host of novel antimicrobials and other

bioactive substances, and similar possibilities exist for nonribosomal polypeptide synthases (Valéry de Crécy-Lagard, Institut Pasteur, Paris, France).

Although the nominal targets of existing antibiotics are now known, it is becoming clear that the successful ones can bind to multiple targets simultaneously – there are at least a dozen penicillin-binding proteins, while fluoroquinolones can bind both to DNA gyrase and to topoisomerase IV. Thus, care may need to be taken if an individual target for a novel antimicrobial drug is sought, and the success of multidrug therapy in treating mycobacterial disease, and the presumed decrease in the likelihood of resistance emerging from such a strategy, both favour the multiple-warhead approach to antimicrobial chemotherapy. But one thing is clear – we are in urgent need of new targets for new antibiotics.

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Biotechnology for world food security

The Michigan State University project 'Agricultural Biotechnology for Sustainable Productivity' recently organised a conference at Asilomar, a location on the Pacific coast with a very special meaning to molecular biologists. This is where, in 1974, many scientists came together for the first time to think about the social implications of gene technology. This time, the topic was limited to applications of modern biology to crops growing in the tropical regions of the world. It is striking how, in 23 years, gene technology has become daily routine in plant breeding, while some applications considered useful at that time, such as transferring the ability to fix nitrogen to cereals, have remained pipedreams. About a third of the participants of the meeting were workers in agricultural research from less-developed countries (LDCs). The debates at the meeting went beyond the strict realm of science and touched on technology

transfer and the consequences of modern plant breeding for LDCs.

The world food problems are daunting: the United Nations Food and Agricultural Organisation estimates that, today, 800 million people do not have enough to eat, while the world population is increasing by around 80 million each year, with most of this increase in LDCs. New plant varieties may make it possible to improve food security without using more farm land, water or other scarce resources. It must be stressed, however, that new technologies alone are not sufficient. Other key issues are good government, access to microcredits for buying seed and fertilizer, fair prices for farm products, efficient transport systems to move produce to consumers and better education (particularly for women). I will cite only some selected examples of how various techniques of modern biology are applied to improve crop plants.

Bananas as a staple food

Bananas are by no means grown only as a cash crop, as we, living in the North, may think. In fact, only 10% of the world production (total 50 million tons yr⁻¹) are exported, the rest being consumed locally. As Oscar Arias (Agrobiotecnología de Costa Rica, Alajuela, Costa Rica) reported, banana plants are very susceptible to diverse diseases and pests, and therefore traditionally require a heavy input of agrochemicals. These are costly, and may be dangerous to the workers handling them if they are not well trained. In the past ten years, methods have been developed and applied in Costa Rica to propagate bananas asexually in the laboratory and in more-or-less sterile glass houses, so that the young plants are free of pathogens and, when they are about 30 cm high, can then be transferred to the field in a healthy state. With this head start, the plants bear fruit in the field after only nine months and then again every four months for many years. Even so, two fungal diseases still cause very serious problems. In the laboratory, several methods of micropropagation and of callus or cell culture are used to help find less susceptible strains of banana.