Lessons from the Fersht laboratory could be vital for the future of the pharmaceutical industry

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Introduction

I enjoyed a wonderful experience, working in the Fersht laboratory from 1984 to 1987. Sir Alan and the other staff he recruited were not only brilliant, innovative scientists, but also warm, friendly and highly supportive people. His leadership style meant that work was always great fun and the excitement of gaining fundamental insights from cross-disciplinary science made it easy to commit time and energy. Outside the laboratory, it is perhaps not widely known that the group enjoyed practical jokes, satire and other humour. Staff were relaxed and always ready to help each other. Sir Alan has a broad range of interests in addition to science and his genius always shines through. During the evening at a Harden Conference on protein engineering, Sir Alan joined in a general knowledge quiz with group members. The only problem was that Sir Alan knew so many answers, which meant he repeatedly asked the next question and the other players had no chance of winning.

The title of Sir Alan’s latest book, ‘Structure and Mechanism in Protein Science’, captures the essence of his huge contribution to the field. He not only showed how enzymes function as chemical catalysts, but also brought highly relevant experimental measurements to the previously largely theoretical topic of biomolecular recognition and then continued by making breakthroughs in our understanding of protein folding.

The output of the Fersht group already is well documented. This article aims to take a different perspective. The invention of new medicines is of considerable importance to society. I believe that the excellent science of the Fersht laboratory illustrates ways in which drug discovery research can be improved. After working in Sir Alan’s group, I joined the pharmaceutical industry. In this article, I use examples to suggest how lessons from the Fersht laboratory can be deployed for the benefit of drug discovery.

The problem

Drug discovery requires the solution of multiple, complex scientific problems. Accordingly, large pharmaceutical companies have a history of high costs and low success rates. Despite these challenges, the industry has been highly profitable due to the launch of sufficient numbers of new medicines. However, the problem is that some observers fear that the future of many companies is under threat, because fewer new medicines are being approved by the regulatory authorities (see Paul et al., 2010). This would have damaging effects on human health, the finances of many countries and scientific innovation.

I have been fortunate to work with excellent colleagues, both in the Fersht laboratory and the pharmaceutical industry. This experience has given me a perspective across several drug discovery organisations. My information derives from direct experience, personal communications and publications. This article outlines why I have the opinion that the invention of new medicines would be more successful if the industry more widely deployed the ‘Fersht Principles’. However, these methods do not address all of the challenges, because development of new medicines is a highly complex process.

Approaches to address the problem

Many large pharmaceutical companies often treat drug discovery as a coordinated sequence of processes, with less focus on developing insight in order to overcome scientific and clinical problems (see Lundqvist, 2005). Experimental design usually is driven by the feasibility of collecting large quantities of data, with less importance attached to the interpretability and relevance of the results. The Fersht laboratory approach combines structure and mechanism studies designed to give insight and solve problems. It is focused on a small number of measurements making it faster and more economical. The pharmaceutical industry often uses structural data, but frequently gains only a fraction of the potential value because it is not combined with mechanistic studies. Mechanistic approaches used in the Fersht group include enzyme kinetics, biophysical measurements, physical organic chemistry and protein engineering—the analysis of site-directed mutants that have been designed using structural data. In order to obtain similar insight, medicinal chemistry needs to add mechanistic studies to enhance the conventional
approaches of structure-based compound design, synthesis and evaluation.

Industry usually deploys a stepwise procedure, first obtaining compounds with good potency in vitro and then optimising their clinical efficacy. However, the strong activity in vitro often is linked with excessive lipophilicity and molecular weight, which compromise key clinical requirements such as bioavailability, selectivity and efficacy (Leeson and Springthorpe, 2007). Some of these issues are overcome by the recent emergence of fragment-based drug discovery (Hajduk and Greer, 2007), which uses similar strategies to the Fersht laboratory in that it involves information-rich experimental designs (fragments as efficient probes of chemical space) in combination with 3-D structure data and precise biophysical measurements.

Elucidation of mechanism

Drugs require high binding affinity. Work in the Fersht laboratory on tyrosyl-tRNA synthetase (TyrTS) demonstrated how a loop, mobile and so invisible in crystal structures, is involved in extremely tight binding in the transition state (Leatherbarrow et al., 1985). This exemplifies the importance of combining studies on mechanism and structure. Medicinal chemists tend to consider 3-D structures as rigid coordinates from crystallography and characterise compounds using the concentration required for 50% inhibition (IC_{50}). This can be very deceptive as binding usually involves an induced fit (Teague, 2003) and the magnitude of IC_{50} usually depends on the assay conditions (Roberts and Ward, 2002). This focus on IC_{50} values can result in misleading estimates of potency and selectivity, which are used as key measurements in the selection and optimisation of lead compounds. IC_{50}s do not take into account how potency is influenced by the mechanism of inhibition, the K_m value and the substrate concentration, which often changes between experiments and on moving from isolated protein assays to the disease state (Roberts and Ward, 2002; Copeland, 2005). Knowledge of mechanism of inhibition helps to account for these confounding factors and permits the prediction of potency under physiologically relevant conditions.

Work in the Fersht laboratory has demonstrated the importance of conformation changes during the catalytic cycle, illustrated by comparison of the structures of free TyrTS and its complexes with Tyr and tyrosyl adenylate intermediate (Fersht, 1987). Many enzymes follow mechanisms where conformation changes mean that prior association with one type of substrate (or coenzyme) is required before binding of a different substrate. Similarly, the immunosuppressant drug, mycophenolic acid, acts by binding to its target enzyme, inositol monophosphate dehydrogenase, only after formation of a catalytic intermediate (Sintchak, et al., 1996). Industrial enzymologists have highlighted how many hit identification assays focus on only a subset of enzyme forms (often free enzyme), which may have limited relevance in disease because of their low abundance (e.g. due to near saturating concentrations of substrates or products) (Copeland, 2005). This emphasis limits the diversity of the hits, decreasing options for lead optimisation, compromising success rates and making it more difficult to secure patent protection for novel intellectual property. Conversely, information on enzyme mechanism can be used to design hit identification assays to contain a balanced mixture of relevant enzyme forms (e.g. complexes with substrates, intermediates or products) and so increases the utility of the output (Copeland, 2005).

Calculation of kinetic parameter values

Rigorous data analysis is essential to characterise enzymes, substrates and inhibitors. The then revolutionary nonlinear regression software, Enzfit, was developed by Robin Leatherbarrow when working in the Fersht laboratory in the early 1980s and was subsequently enhanced to give GraFit (Erithacus Software Ltd, see www.erithacus.com). The methods deployed in the Fersht laboratory ensured the calculation of relevant and reliable kinetic parameter values. Although the pharmaceutical industry now routinely applies nonlinear regression algorithms similar to that in GraFit, there sometimes is insufficient regard as to whether the deployed dose–response equation relates to the physical processes in the assay, whether it gives an adequate quality of fit, whether it contains redundant variables, or whether there is a low degree of precision (high standard error) in the estimated parameter values. Accordingly, company databases contain many IC_{50} values which do not correlate with the true potency of the compound due to common issues such as nonspecific (superstoichiometric) inhibition, tight binding (depletion of free inhibitor by binding to target protein), slow binding (IC_{50} varies with time) or assays containing a mixture of enzyme forms (phosphorylation states, redox states, products of partial proteolysis) with differing sensitivity to the test compound. The standards of data analysis in the pharmaceutical industry perhaps could be improved by ensuring use of an appropriate dose–response equation and monitoring quality of fit (O’Shannessy, 1994; Roberts and Ward, 2002; Motulsky and Christopoulos, 2003).

This article has focused on mechanistic enzymology and the development of enzyme inhibitors as drugs. Future medicines also will treat diseases caused by aberrant protein folding. Sir Alan Fersht also is a leader in protein folding and his work in this area will be of fundamental importance.

Conclusions

Large pharmaceutical companies are achieving insufficient success, despite strong investment in technology, organisation and efficiency. Work in the Fersht laboratory highlights how progress in research requires rigorous experiments designed to give mechanistic insight. I believe that, by increasing the use of these approaches, the industry would generate better-optimised candidate drugs and so help to solve the key problem—the low success rate in clinical development.

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Conflict of interest: I offer consultancy and training on the identification and evaluation of enzyme inhibitors in drug discovery.
References