A structure-based approach to drug discovery; crystallography and implications for the development of antiparasite drugs

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SUMMARY

Advances in the life and physical sciences have enabled us to characterize the 3-dimensional structure and the biochemical or biological activity of both small and large molecules. The use of structural chemistry to assist understanding of biological activity provides information relevant to the design, development or identification of new pharmaceuticals. This structure based approach has become an important component of drug research and is in widespread use by the major pharmaceutical companies. A brief historical introduction, to convey how this area of science has reached the present stage, is given. The basis of the structural approach to understanding the chemistry of small and large molecule biological activity is outlined with an emphasis on the use of results derived from X-ray diffraction methods. Developments in other areas are discussed to emphasize the multidisciplinary nature of this research and the benefits of combining different methods. Examples of protein crystallographic studies in the area of molecular parasitology, some of which are directly relevant to antiparasite drug design, are presented. The characterization of the enzyme trypanothione reductase, a project which has benefited from many of the recent developments, is detailed. Future challenges and difficulties, both scientific and economic, are discussed.

Key words: crystallography, drug discovery, enzyme structure, parasitology, trypanothione reductase.

AN HISTORICAL INTRODUCTION

The pharmaceutical industry was established and shaped by several notable events and scientists with the contribution and genius of Paul Ehrlich (Travis, 1991) being foremost in this area of science. Ehrlich recognized, from experiments testing the biological stain methylene blue as an antimalarial, that small molecules could be used to treat infections by pathogens. He gained immensely from developments in the dye industry and his realization that small molecules could be used in this way provided a powerful stimulus to medicinal chemistry. He identified trypan dyes and arsenical compounds as possible treatments of trypanosomal infection. The arsenical compounds were subsequently to provide him with a treatment for syphilis and some arsenicals are still used in the treatment of trypanosomiasis.

Other significant developments were the first screens of soil samples for disease-fighting agents initiated by the microbiologist Rene Dubos in 1927 and in the following year Alexander Fleming found penicillin. Such events encouraged Selman Waksman to carry out a planned search for useful bacterial products, research that identified the widely applicable antibiotic streptomycin (Werth, 1994). Gerhard Domagk in 1935, more in line with the Ehrlich approach, determined that a sulphonamide dye isolated from coal tar was a potent antibiotic effective against streptococci (Mandell & Sande, 1990). Such events served to guide the drug companies' research strategy against what were considered the main medical problems of the time, namely bacterial and viral infections.

The emphasis was on small molecules. The pharmaceutical companies began collating soil samples from throughout the world, identifying and isolating many chemicals and a few useful drugs. These chemicals were characterized, screened for biological activity and, where possible, synthesized. As the methods of analytical and synthetic chemistry developed, strategies for characterization and systematic modification of molecules were applied in the search for more and better drugs. In particular, known metabolites or the constituents of biomolecules were selected for derivatization. An early success was the synthesis of 5-iodo-2′-deoxyuridine by William Prusof in 1958. This thymine derivative inhibits DNA polymerase. Whilst searching for a treatment of ophthalmic viral infections in 1962, Herbert Kaufman identified this compound as a suitable inhibitor of viral DNA polymerase. This led to the first successful antiviral drug for the treatment of Herpes simplex keratitis (Kaufman, 1993). Also of note are the studies of Sir James Black who was characterizing adrenaline and histamine systems (Black, 1989).

The combination of screening and synthesis became the tried and trusted method. The screens were to identify lead compounds with the required biochemical properties. Synthetic chemistry was subsequently brought in to modify the lead with the

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aim of improving the pharmacokinetics, efficacy and bioavailablity. Spectacular advances in the life sciences allowed for improved biochemical and biological knowledge into the 1970s. The Nobel laureate George Hitchings summarized the feeling of the time when he wrote 'To the biochemical chemotherapist, it is not only a matter of faith, but an obvious fact, that every cell type must have a characteristic biochemical pattern and therefore be susceptible to attack at some locus or loci critical for its survival and replication' (Hitchings, 1969).

The search for appropriate cellular targets and the complete biophysical characterization of these molecules has become a focal point in drug research. Our knowledge of the human immune system and metabolic biochemical pathways has provided many clues for appropriate molecular targets, examples of which are hormone or cytokine receptors on a cell surface, DNA/RNA and enzymes. For invasive agents it is particularly helpful if there are differences between the infectious agent and the infected. The ideal target would be a soluble enzyme unique and essential to the pathogen. Advances in cell and molecular biology, microbiology, biochemistry and recombinant DNA technology allow characterization of molecular targets. Drugs are also molecules, often quite simple and small in comparison to the biochemical target, which act as selective poisons able to exert a biological effect by stimulating or inactivating some metabolic pathway. They vary in size, shape, chemical and physical properties. The modern approach to drug discovery is to identify and characterize a biochemical target with respect to a specific ailment and match the target with small molecules that have the right properties to inactivate it.

Developments in a number of disciplines, each interacting with the other, have been instrumental in supporting this search for new pharmaceuticals. I shall consider the developments and applications under the following broad headings: (i) chemistry; (ii) cell and molecular biology; (iii) structure determination of macromolecules; (iv) bioinformatics and computational methods. In every aspect, advances in technology have played critical roles in developments.

Chemistry

A major asset of any pharmaceutical company is its stock of many thousands of small molecules used to screen for biomedical uses. The libraries consist of molecules isolated from plants, soil extracts, mould or bacterial cultures. Synthetic modifications of these molecules have been prepared to increase further the diversity and the numbers of compounds. Improved methods for the isolation, purification and characterization of natural products and for synthesis have greatly expanded these chemical databanks. With the advent of combinatorial chemistry, even larger databases of chemicals for screening are becoming available. Extensive libraries based on peptides, nucleotides, phage-display systems and more recently small organic molecules are being used (Mitchison, 1994; Nielsen, 1994). These molecules can all be used for screening purposes, then stored for future screens as and when new biomolecular targets are identified. The libraries also expand as structural information from biophysical studies or suggestions from molecular modellers are taken up by the synthetic chemists. In the course of characterizing the chemistry of the molecules (natural or synthetic), the structure will have been determined, often by X-ray methods, and so together with the compound libraries we have databases of structural information. Such databases include the Cambridge Crystallographic Databank (Allen & Kennard, 1993) (a depository for all small molecule crystal structures with more than 130000 entries of organic and organometallic compounds) and much larger databases can be constructed on the basis of structural formulae (Borman, 1992).

Note, also, that the development of methods for oligonucleotide synthesis has had a profound influence on molecular biology. Synthetic chemistry can, in addition, allow one company to circumvent the patent rights of a competitor by making modifications to known drugs.

Cell and molecular biology

Microbiologists have improved techniques and equipment for isolating and culturing cells and pathogens whereas protein and natural product chemists also have better methods and equipment for the isolation, sequencing and chemical characterization of large and small molecules. Advances in chromatography, sequencing and mass spectroscopy are particularly noteworthy. Researchers in molecular biology now have access to synthetic DNA and improved methodology, such as the polymerase chain reaction, to assist in getting genes cloned and subsequently overexpressed in bacterial, yeast or other systems together with a number of tricks to ensure that the desired products are correctly folded or readily purified. These include expressing glutathione-S-transferase or maltose-binding-protein fusion systems and co-expressing in the presence of chaperonins (Cole, 1996). Cell and molecular biology, biochemistry, and recombinant DNA technology are critical for the identification, isolation and characterization of molecular targets that can then be sequenced, cloned, overexpressed and purified in large quantities. This provides information for comparisons based on sequence homology and the material for use in biophysical studies to determine the structure. It can also provide a cost-effective way

to obtain material for bio-assays in screening of compound libraries.

The synthetic chemists have improved reagents and reactions for difficult stereo-specific syntheses. This can involve the use of enzymes, generated through the use of recombinant DNA techniques, in the synthetic method. For example, using aldolases to synthesise chiral antibiotics (Wong & Whitesides 1994).

Recombinant methods can provide the large quantities of sample required for a structure determination. They can also enable domains to be isolated from larger protein entities for characterization. Such an approach has proved particularly useful in the characterization of the catalytic domains of collagenases. This is a prelude to inhibitor development in the rational design of new drugs against tissue degradative diseases (Blundell, 1994).

Structure determination of macromolecules

Knowing the 3-dimensional structure of a target can contribute significantly to understanding where and how a biochemical process can occur. The structural chemistry of the active site of an enzyme can be dissected and used to design or identify chemicals that block the necessary interactions for activity. Characterization of enzyme–substrate or enzyme–inhibitor complexes can suggest alterations to improve inhibition. This characterization of the molecular target is pivotal to the whole structure-based design/identification process and it is also important to have reliable structural information on the small molecules that provide the actual drugs or lead molecules. Protein structure determination can involve Nuclear Magnetic Resonance (NMR) spectroscopy (Wuthrich, 1995) and X-ray diffraction methods (Perutz, 1992). Larger, more powerful NMR magnets have assisted the increase in size of proteins that can be studied in solution and the decrease in time necessary for the experiments. Isotope labelling of proteins or inhibitor molecules further aids this analytical tool. Even so, this technique is still limited in application to highly soluble proteins with a molecular weight of around 25 kDa. Crystallography remains the method of choice to derive accurate 3-dimensional models for most proteins.

Tens, occasionally hundreds, of milligrams of highly purified protein are required in order to achieve a crystallographic structure determination. Well ordered crystals of around 0·1–0·5 mm must be obtained and this involves empirical testing of conditions designed to bring about an ordered precipitation. This stage can present a significant hurdle and a great many projects fail at this point. Recombinant methods allow for the provision of the necessary quantities of protein that are required to test for crystal growth. The explosion in the number of protein crystals structures that are being reported is in large measure due to advances in molecular biology. It is not only quantity of a given sample that is an issue. It is possible to obtain the same enzyme from different organisms (this can be an important variable in crystal growth), to engineer changes in amino acid residues to promote molecular aggregation and perhaps crystallization, to isolate domains of interest, to improve protein isolation and purification by using fusion systems involving, for example, glutathione-S-transferase and to label methionine residues with selenium to assist in structure determination.

Improved X-ray generators and detector systems, including the use of synchrotron radiation, have greatly influenced the speed and accuracy of crystallographic studies. In particular, synchrotron radiation can be orders of magnitude more intense than the X-rays obtained in the home laboratory. In combination with more sensitive area detectors this allows for enhanced rates of data collection. Synchrotron radiation can be tuned to provide X-ray wavelengths beneficial to structure determination using anomalous dispersion effects. This is particularly useful when applied to selenomethionine-labelled proteins, a method becoming increasingly popular. Higher order, improved quality diffraction measurements can be made that, in turn, can improve the accuracy of the structural models. Accuracy and reliability are important in determining protein structures. After all, we need to be confident about the structural details, for example the patterns of hydrogen bonding, of our drug targets.

Bioinformatics and computational methods

Greatly enhanced computational facilities (software and hardware) have contributed to speeding up structural analyses and to improving the quality of structures being determined. Efficient computers allow for the storage and handling of immense catalogues of information. This encompasses physical, biochemical and structural detail of small and large molecules. Graphics workstations enable scientists to visualize complicated molecules in 3-dimensions and allow molecular docking methods, simulation experiments to investigate different molecular conformations, and modelling whereby predictions of protein–inhibitor interactions can be made to suggest new experiments. Developments in software now provide improved visualization programs, database retrieval and search procedures to aid the molecular modeller.

Even without chemical leads, if the structure of the molecular target is known and, in the case of an enzyme, the active site chemistry is well defined, it is possible to map out the properties of the active site. This is achieved with a wide range of computer

programs (Borman, 1992; Kuntz, Meng & Shoichet, 1994). This can define a template with which to search databases of small molecule structures to locate molecules that might have the size, shape and electrostatic properties to bind and inhibit the target. Modern computational chemistry will allow the 2-dimensional structural formulae to be turned into a 3-dimensional shape which can be stored in a database. The size of such a database can be enormous and in general decisions are taken to use subsets of chemicals selected on specific criteria such as ease of synthesis, biological activity, toxicity and the like.

Molecular biology is providing more gene–amino acid sequences than we have structures for. We already know that previously determined structures, which may have no apparent therapeutic value, can provide critical information to be used in the modelling of target proteins or, if the active site and enzyme mechanisms are similar, to aid inhibitor characterization. For example, two zinc proteinases, thermolysin (Colman, Jansonius & Matthews, 1972) and carboxypeptidase A (Lipscomb, 1970), which are amongst the first enzyme structures determined by crystallographic methods, have proved useful in this respect. These enzymes are related to matrix metalloproteinases such as collagenase (Blundell, 1994) and to angiotensin converting enzyme (ACE) (Hassell *et al*. 1984; Perutz, 1992) which are important therapeutic targets. Thus two examples of protein structures determined out of curiosity (basic research!) have provided important information for use in applied science. Structural information from the thermolysin-inhibitor studies guided work by Hassell and colleagues on ACE. They had only to synthesize and test fewer than 100 compounds to identify a drug for the treatment of hypertension called Cilazapril (Hassell *et al*. 1984; Attwood *et al*. 1986).

Advances in homology modelling allow for the construction of models of the target proteins if a related structure has previously been determined. A recent example is the computer modelling of a cysteine proteinase from *Plasmodium* and the use of this model to identify inhibitors that provide leads for antimalarial drugs (Ring *et al*. 1993). Since crystallography and NMR studies are producing more and more structures, there is an ever increasing chance that an appropriate starting model can be found. As of July 1996, there were in excess of 4600 macromolecule structures stored in the Protein Databank at Brookhaven. This represents an extremely valuable store of molecular data.

Sequence analysis is increasingly allowing for domain or module identification and provides information to use in searches to find families of protein folds (Orengo, Jones & Thornton, 1994). Such advances will support the computer graphics modelling of new structures.

STRUCTURE-BASED DRUG DISCOVERY, THE STRATEGY AND SOME EXAMPLES

If successful, the structural analysis provides an accurate 3-dimensional picture of the target molecule and perhaps also details of substrate or inhibitor complexes. This can enhance understanding of chemical reactivity and provide clues for inhibitor design. Studies on the enzyme thymidylate synthase, a target for anticancer therapy, provide an excellent example of this (Shoichet *et al*. 1993). The structural detail can help identify a common pharmacophore (the chemical framework necessary for the biological activity) and indicate where chemical interactions responsible for binding occur (the donor and acceptor groups that form hydrogen bonds, charged groups to promote electrostatic interactions, van der Waals' interactions, aromatic rings or other distinctive shapes). This detail can assist molecular modellers to design molecules with similar structures to the inhibitors or substrates but with specific alterations to improve binding. A further round of structural studies can ascertain whether the modelling presumptions were correct and what more in the way of chemical modification can be done to improve binding.

The very exciting study involving influenza virus neuraminidase (von Itzstein *et al*. 1993) adopted such a strategy. Neuraminidase is located on the surface of the influenza virus. The enzyme cleaves the virus from the infected cell providing a release mechanism and allowing the spread of the virus. If release was inhibited then this might control infection. Neuraminidase has been characterized by X-ray crystallography (Varghese & Colman, 1991). The structure of the enzyme in complex with a transition state analogue suggested how improved interactions could be built onto the neuraminic acid unit with the result that a compound with an nM binding constant was identified and has gone forward to clinical trials (von Itzstein *et al*. 1993).

The link between structure determination and making use of the results has become an important part of drug discovery. A number of excellent reviews outlining strategies and listing many specific projects have been produced (Hol, 1986; Perutz, 1992; Bugg, Carson & Montgomery, 1993; Colman, 1994; Johnson, 1994; Kuntz *et al*. 1994; Verlinde & Hol, 1994). The surge of interest is such that almost monthly new structures of therapeutic targets implicated in a wide range of diseases are being published. Studies underway involve the HIV integrase fragment (O'Brien, 1994) complementing previous and extensive structural studies of two other HIV drug targets, a proteinase (Desjarlais *et al*. 1990; Fitzgerald, 1994) and reverse transcriptase (Kohlstaedt *et al*. 1992).

It is also important to identify how drugs that are already in use can function. An early example of this

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was the enzyme dihydrofolate reductase (DHFR) and the inhibitor methotrexate. DHFR reduces dihydrofolate to tetrahydrofolate which is essential for the synthesis of thymidine and hence is important in cell division. So inhibition of DHFR prevents the synthesis of new DNA, hence this enzyme represents a target for anticancer, antibacterial, antiprotozoal and antifungal drug development (Elion, 1989; Hitchings, 1989) A structural explanation of how this drug inhibits DHFR is provided by the crystal structure of the enzyme–inhibitor complex (Bolin *et al*. 1982). Another example concerning an anti-parasite drug will be given later.

The use of traditional screening methods continues. It would be nonsensical to stop testing the databanks of compounds given improvements in the screening processes and, of course, the availability of more targets to study. The ability to combine screening results that identify some molecule of interest with structural studies to determine the chemistry behind a biochemical action promises to provide a lot of useful data. From this, the search begins for modifications to make a clinically useful drug.

Fortuitous observations by prepared scientists will continue to assist drug development. A recent example from the (formerly) Wellcome Research Laboratories is Lamotrigine (Peck, 1994). From the observation that patients with chronic epilepsy often had macrocytic anaemia due to folate deficiency, a link between folate metabolism and seizure control was established. This occurred within the company who knew more about folate metabolism, via their interest in developing cancer treatments and also antiparasite drugs (Elion, 1989; Hitchings, 1989), than any other. So a known DHFR inhibitor, pyrimethamine, was successfully tested for anti-convulsant properties, then chemically modified to produce the derivative called Lamotrigine. This compound is proving to be a useful broad-spectrum anticonvulsant with improved pharmacokinetic properties compared with many other compounds in use.

ECONOMIC CONSIDERATIONS

On scientific grounds the development of a more direct, less random approach is logical. However, economic arguments have a bearing on the situation. It has been estimated that if between 6000 and 10000 compounds are screened, maybe 10 will provide pharmacological activity (Vagelos, 1991; Gutteridge, 1993; Billstein, 1994). Some disasters in the application of therapeutic agents, notably contaminated sulphonamides in the 1930s in America (Werth, 1994) and thalidomide in the late 1950s, have contributed to tight legislation that controls the testing and evaluation of drugs. This is necessary legislation which does, however, place a further time restraint on pharmaceutical companies. So, even after finding a suitable compound and patenting it, there are the pre-clinical trials that take between 2 and 4 years followed by the 4–6 years required for the 3 distinct stages of clinical assessment. As medical diagnostics improve with technological advances, this time period may well be reduced. Nevertheless, out of the 10 active compounds maybe only 1 will pass the necessary clinical/safety trials and reach the market place.

There are many reasons why a chemical may prove unsuitable for development as a therapeutic agent (Nies, 1990). These range from toxicity, poor efficacy, poor retention time in the patient, instability, low bioavailability to high cost of synthesis. The identification and clinical trials of the drug may have taken 12 or more years and cost in excess of £100 million. Since a patent in the USA lasts 17 years, the company may have a relatively short timescale in which to make a profit. It should be noted that even should a drug reach the market place, it may not make a profit (Vagelos, 1991). Whereas a rational approach to drug discovery is attractive to the companies because it promises to accelerate the drug discovery process, the need for different and expensive technology suggests that costs associated with research and development will if anything be increased. Despite this, because the benefits warrant the cost, the major companies have moved towards a multidisciplinary structure-based approach in the search for new, more effective drugs.

It remains an unfortunate fact that most prevalent parasitic infections and subsequent afflictions are of no interest to the major pharmaceutical companies. How ironic given that the seminal contributions from Paul Ehrlich derived from studies on tropical parasites. The financial justification for this situation is clear. Human and veterinary medicine in developing countries (where the most widespread parasitic infections occur) cannot provide the desired profits. In terms of parasitology, it appears that academic research groups are the ones striving to make progress.

CRYSTALLOGRAPHY APPLIED TO PROTEINS FROM PARASITES

Advances and developments in cell and molecular biology have influenced and contributed to parasitology so that molecular parasitology now exits as a sub-discipline in its own right (Fairlamb, 1989; Hyde, 1990). Where molecules are involved there is a need to know about structure and since the organisms and their metabolic pathways are of medical interest there is scope for structure-based drug design. Parasite protein structures that have been determined or for which crystals have been

Table 1. Parasite proteins characterized using single crystal X-ray diffraction methods or for which crystals have been reported*

Protein	Species	References
Chymotrypsin/elastase inhibitor	*A suum*	Huang *et al*. 1994
Cruzain	*T. cruzi*	McGrath *et al*. 1995
Dihydrofolate reductase–thymidylate synthase	*L. major*	Knighton *et al*. 1994
Glutathione-S-transferase	*S. mansoni*, *S. japonicum*	Lim *et al*. 1994; Trottein *et al*. 1992; McTigue *et al*. 1995
Glyceraldehyde-3-phosphate dehydrogenase	*L. mexicana*, *T. cruzi*, *T. brucei*	Kim *et al*. 1995; Oliva *et al*. 1996; Van Calenbergh *et al*. 1995
Haemoglobin	*A. suum*	Yang *et al*. 1995
Hypoxanthine–guanine phosphoribosyltransferase	*S. mansoni*, *T. foetus*, *T. gondii*	Focia *et al*. 1996; Somoza *et al*. 1996; Schumacher, Carter, Ullman & Brennan, pers. comm.
Inosine-5′-monophosphate dehydrogenase	*T. foetus*	Whitby *et al*. 1995
Lactate dehydrogenase	*P. falciparum*	Dunn *et al*. 1996; Chattopadhyay *et al*. 1996
Leishmanolysin	*L. major*	Schlagenhauf *et al*. 1995
Nucleoside *N*-glycohydrolase	*C. fasciculata*	Degano *et al*. 1996
Ornithine decarboxylase	*T. brucei*	Grishin *et al*. 1996
$p12^{cks1}$	*L. mexicana*	Mottram & Grant, pers. comm.
Phosphoglycerate kinase	*T. brucei*	Bernstein *et al*. 1997
6-phosphogluconate dehydrogenase	*T. brucei*	Barrett *et al*. 1994
Pteridine reductase	*Leishmania* sp.	Hardy & Beverley, pers. comm.
Triosephosphate isomerase	*T. brucei*	Verlinde *et al*. 1992
Trypanothione reductase	*C. fasciculata*, *T. cruzi*	Zhang *et al*. and references therein 1996
Variable Surface Glycoprotein	*T. brucei*	Blum *et al*. 1993

* The proteins are listed in alphabetical order and the reference given may not be the first report of a structure determination. The most recent reference available is provided.

reported are listed in Table 1. As already outlined, we now have access to recombinant proteins which can circumvent the problems of parasite growth and maintenance prior to protein isolation and purification and the difficulty of obtaining sufficient protein. Most of the entries in Table 1 involve studies from recent years involving just this approach. More structures of proteins from parasites will undoubtedly follow rapidly as further inroads into parasite biochemistry and molecular biology are made. Developments in parasite signalling in particular look to be a promising area in which to identify therapeutic targets (Boshart & Mottram, 1997). Not all of the structures listed in Table 1 represent ideal targets for drug design but they all have a story to tell! Several will now be detailed to provide an overall picture of the area.

One of the first parasite proteins to be characterized was the variable surface glycoprotein from *Trypanosoma brucei*. Although not a drug target, this is a most beautiful example of a 3-dimensional structure providing information relevant to a biological question. In this case the structure shows how the African trypanosome is able to outwit the immune system (Blum *et al*. 1993). The glycosomal enzymes from trypanosomes are being characterized and inhibitor design projects are underway (Hol, 1986; Verlinde & Hol, 1994). Trypanosomes have an organelle called the glycosome in which part of the glycolytic process is carried out. Such compartmentalization may assist the efficiency of glycolysis in trypanosomes. Given the high rate of glycolysis in the parasite, compounds able to curtail glycolysis could put the organism under stress. Structural studies on glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase are well advanced and more recently the structure of phosphoglycerate kinase from a trypanosome species has been characterized (Bernstein, Michaels & Hol, 1997). Research on glyceraldehyde-3-phosphate dehydrogenase was described by Verlinde & Hol (1994) and studies on triosephosphate isomerase are detailed by Verlinde *et al*. (1992). These investigations have not only pioneered the concept of structure-based drug discovery against parasitic organisms but have had an impact on the whole field.

Aspects of parasite nucleic acid metabolism look attractive from a drug design aspect. The bifunctional dihydrofolate reductase–thymidylate synthase (DHFR-TS) from *Leishmania* has been characterized. In this case the parasite combines two enzyme activities into a single protein entity. Given the role of both enzymes in DNA synthesis and the wealth of information already generated on both

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DHFR and TS inhibitors, then this parasite enzyme looks a promising target. The hypoxanthine phosphoribosyltransferases (HGPRTs) are involved in the salvage of purine nucleotides. Most protozoan parasites lack the ability to synthesize, *de novo*, purine nucleotides and so the salvage process provides a key metabolic function. Structures of the enzymes from the bovine parasite *Tritrichomonas foetus* and the human parasites *Toxoplasma gondii* and *Schistosoma mansoni* have been produced. There are differences between the human HGPRT and the parasite enzymes that could form the basis of drug design methods. An enzyme also involved in purine salvage is the inosine–uridine nucleoside *N*-ribohydrolase. This enzyme salvages exogenous inosine through a hydrolysis reaction. A recent structure of this tetrameric enzyme shows a novel active site and explains the tight binding of several known enzyme inhibitors (Degano *et al*. 1996). Trypanosomatids are also pterin auxotrophs and the enzyme pteridine reductase which mediates pteridine salvage looks a promising target for drug design (Bello *et al*. 1994).

The cysteine proteinase known as cruzain and the zinc proteinase called leishmanolysin are under investigation. A structure has been reported for the former (McGrath *et al*. 1995). The proteinase inhibitor from *Ascaris* has been characterized in complex with pig elastase. The complex indicates the structural mechanism that allows the inhibitor to prevent the host enzyme from acting on the parasite (Huang *et al*. 1994). A number of structures of glutathione-S-transferase from helminth parasites have been produced. Of particular note is the complex of the enzyme with the leading anti-schistosomal drug praziquantel (McTigue, William & Tainer, 1995). The drug binds at the dimer interface using steric inhibition to exclude substrate. Knowledge of the inhibitor-complex structure provides useful detail that could provide the basis for the design of new potent compounds.

Two enzymes involved in aspects of polyamine metabolism, ornithine decarboxylase (ODC) and trypanothione reductase, are attracting considerable interest. ODC is a pyridoxal phosphate-dependent enzyme that produces putrescine from ornithine. The role of polyamines in regulating cell growth and differentiation is being increasingly recognized and ODC is of interest as a drug target, not only in trypanosomes but also for anticancer use. The organo-fluorine compound α-difluoromethylornithine (DFMO) has proven ability for the treatment of African sleeping sickness, although there are practical limitations. Structural information may assist the development of DFMO derivatives with improved properties.

The trypanothione reductase study is a typical example of a project directed towards drug design. It is further advanced than some of the other projects listed and so it will be detailed here.

TRYPANOTHIONE REDUCTASE

The discovery that trypanosomes use peptide–polyamine conjugate metabolites, such as trypanothione ((*N*^{1}, *N*^{8}-*bis*(glutathionyl)spermidine; Fig. 1) and *N*^{1}-glutathionylspermidine, to regulate the intracellular reducing environment and to protect them from oxidative stress sparked considerable interest (Fairlamb, 1989; Schirmer, Muller & Krauth-Siegel, 1995). These thiols are oxidized to disulphides in the course of this protective role. Trypanothione reductase (TR) maintains the reducing environment in the protozoan cell by reduction of the disulphides to maintain high levels of the protective thiols (Fig. 1). Humans utilize a different, although related, system which has been thoroughly characterized (Schirmer, *et al*. 1995). The protective thiol is glutathione (L-γ-glutamyl-L-cysteinylglycine) which is oxidized to glutathione disulphide and the cognate enzyme is glutathione reductase (GR).

TR and GR are closely related enzymes. They are both NADPH-dependent disulphide oxidoreductases, homodimeric with a subunit molecular weight of approximately 52 kDa and utilize a prosthetic FAD. Although TRs and mammalian GRs share approximately 40% sequence identity and the residues involved in catalysis are conserved, the enzymes are mutually exclusive in respect of disulphide substrate specificity (Fairlamb & Cerami, 1992). Since trypanothione and TR have been observed in all trypanosomatids so far investigated but not in mammalian cells, in theory it should be possible to identify a compound that has the correct chemical properties to bind to and inhibit the function of the parasite's TR but which does not inactivate the host's GR. Such a compound could be used to treat a wide range of trypanosomatid infections, it could disable the parasites' ability to cope with oxidative stress such that it would die or be more susceptible to a host immune reponse or other drugs. The further observation that many chemicals already identified as having anti-trypanosomal properties show activity against trypanothione metabolism, including therapeutic arsenicals and antimonials (Cunningham, Zvelebil & Fairlamb, 1994; Schirmer, Muller & Krauth-Siegel, 1995), make this aspect of trypanosomatid biochemistry an excellent target for the development of new drugs. Screening methods or a rational design process would be applicable and with the latter approach in mind structural detail of TR to provide an understanding of structure-function relationships was sought, with a number of groups addressing the problem (Kuriyan *et al*. 1991; Hunter *et al*. 1992; Lantwin *et al*. 1994).

In collaboration with Alan Fairlamb (then at the London School of Hygiene and Tropical Medicine) we started by investigating *Crithidia fasciculata* TR

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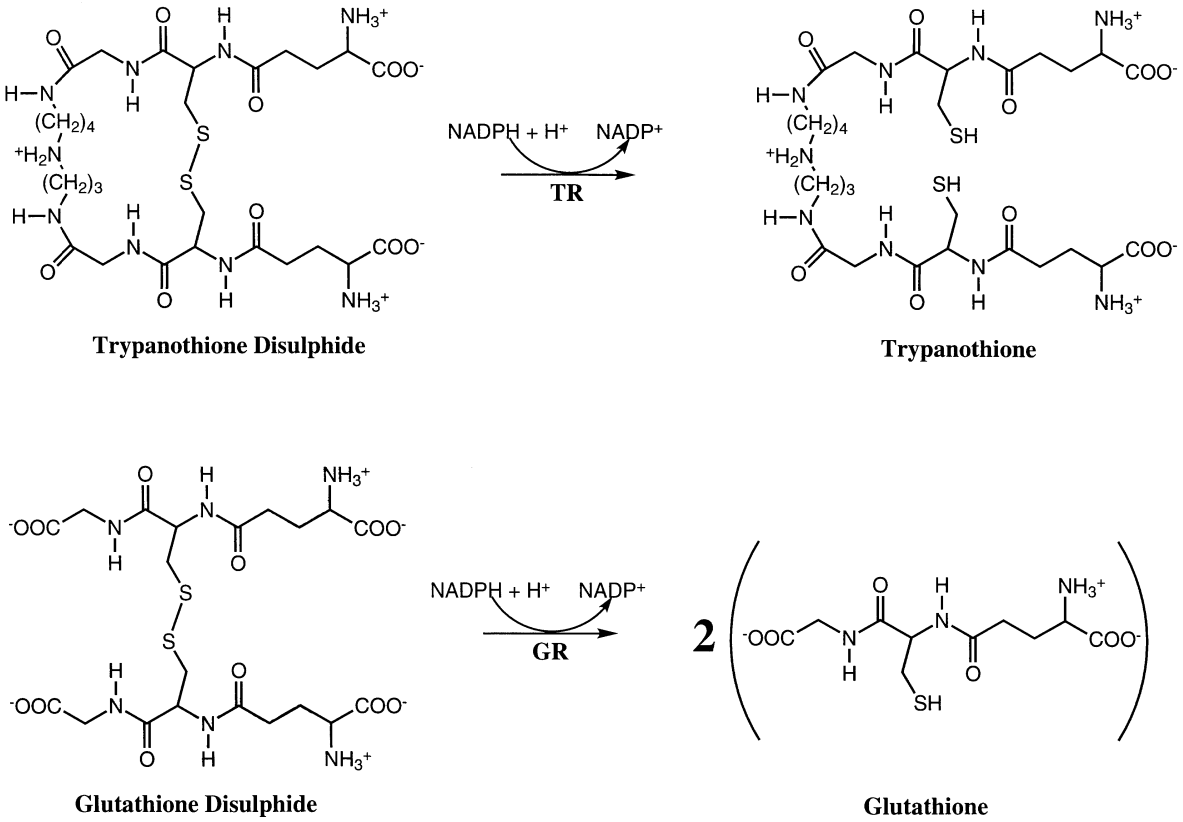

Fig. 1. Reactions in host and trypanosome. (a) The parasite metabolite trypanothione and (b) the human metabolite glutathione, both shown in oxidized and reduced forms.

(Hunter *et al*. 1992). From a few milligrams of purified enzyme we were able to grow a few small crystals. The crystals displayed a unit cell with all cell edges close to or greater than 100 Å and in order to get good enough diffraction data, required to produce an accurate structure, we made use of the intense and well collimated synchrotron facility at Daresbury laboratory. Access to synchrotron radiation has greatly benefited our study. We were able to determine a structure of the enzyme by itself and in complex with glutathionylspermidine disulphide (Bailey *et al*. 1993). From our results we were able to explain the structural basis for TR and GR only processing their own substrates, as discussed below. However, despite our success a number of complications emerged. The low yields of enzyme that could be purified were a limiting factor in terms of quantity of material for further crystallographic study. We also decided it was a priority to characterize the actual target, namely a TR from a human pathogen. So an *Escherichia coli* overexpression system for TR from the causal agent of Chagas' disease, *T. cruzi*, was constructed (Borges *et al*. 1995). This provided large quantities of enzyme for our ongoing studies. Access to such quantities of TR has enabled a rigorous screening of crystallization conditions and this has produced tetragonal crystals. Again, a moderately large unit cell was obtained and using synchrotron radiation we were able to record high resolution data (Zhang *et al*. 1996). We have determined the crystal structure of this TR (Zhang *et al*. 1996; Figs 2, 3) and of complexes of it with substrates. In addition, using recombinant site-directed mutagenesis methods we have been able to produce and characterize the 3-dimensional structure of several altered TR's. This later study is providing details relevant to how the enzyme achieves catalysis. Such information may assist in the development of transition state analogues as inhibitors.

The crystallographic studies on TR and GR explain why each enzyme is so specific for its own substrate. The chemical determinants of this specificity are size and charge. The smaller, negatively charged human substrate glutathione fits neatly into a positively charged cleft on the enzyme surface. In particular, at one side of the cleft a positively charged bank of three arginines interacts with two of the carboxylate groups of glutathione. In contrast, the trypanosomal enzyme presents an enlarged active site cleft that complements the positive charge of trypanothione. The arginines in GR are replaced by a tryptophan, asparagine and alanine. The tryptophan acts in concert with a methionine to create a hydrophobic patch that interacts with the spermidine component of the TR substrates. In addition, a

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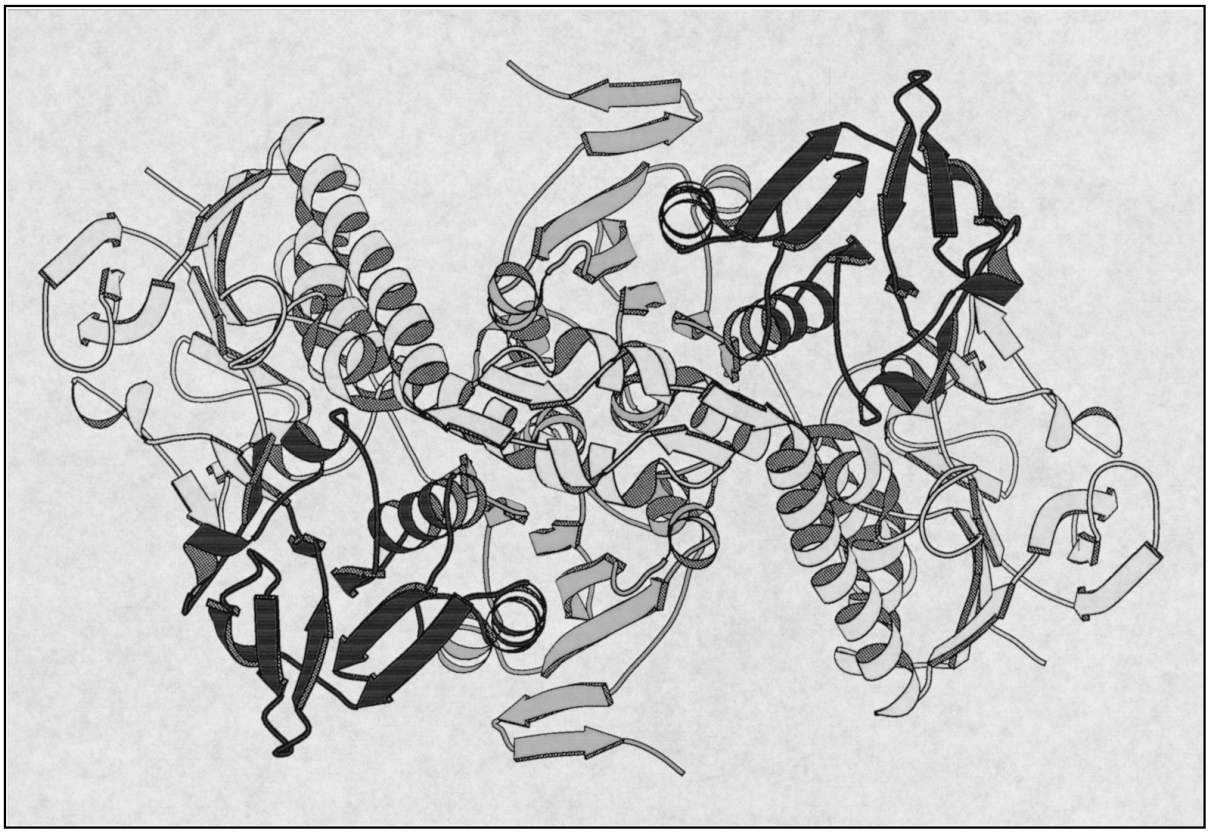

Fig. 2. A schematic diagram of the trypanothione reductase dimer. All side-chains are removed in order to simplify the picture and only main-chain atoms are used to construct a representation of the elements of protein secondary structure. Helices are depicted as coiled ribbons, sheets as arrows. Figs. 2 and 3 were produced using MOLSCRIPT (Kraulis, 1991)

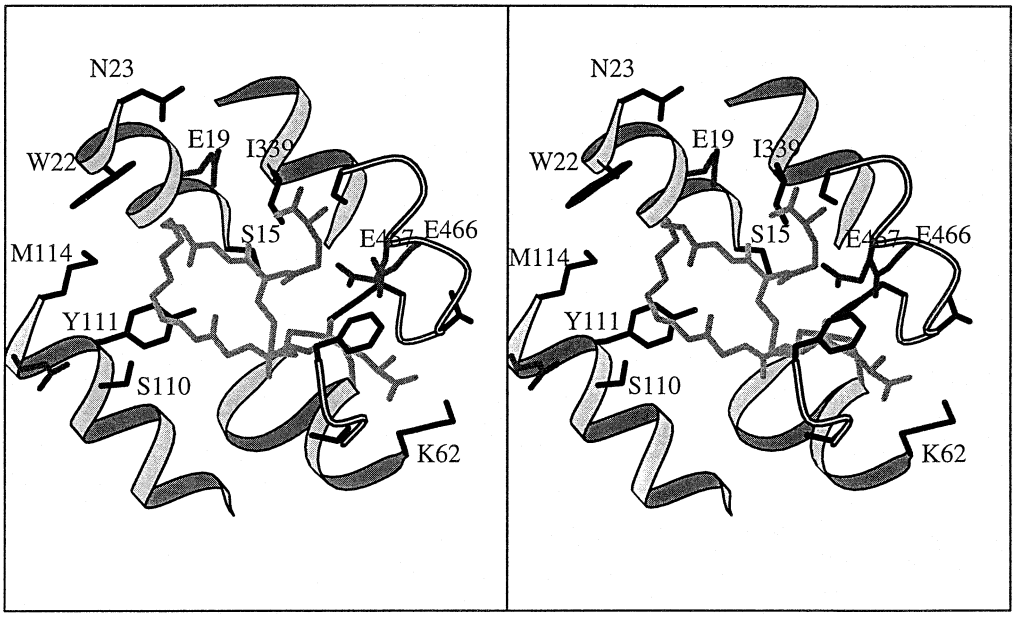

Fig. 3. A stereoview into the active site of trypanothione reductase. Bound trypanothione is shown and key residues are labelled with the single letter code.

negative glutamic acid is positioned to hydrogen bond to an amide group of trypanothione. This glutamic acid and hydrophobic patch in TR would repulse the negative glutathione. The positive arginines of GR would repulse trypanothione and the smaller active site would not allow the parasite substrate to fit into the active site. This structural detail provides information to use in an ongoing

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structure based approach to TR inhibitor design. A range of TR inhibitors have now been identified and are being investigated in a number of laboratories (e.g. Ponasek *et al*. 1995; Schirmer *et al*. 1995).

FINAL COMMENTS

The goals of a rational approach to structure-aided drug design or drug discovery are well defined. Our knowledge of biochemistry, immunology and molecular biology is increasing at a phenomenal rate and the research efforts to find new drugs are supported by immense advances in technology. A decade ago we had the structures of only a few targets, limited small molecule databases and much to learn. Today the field, although still immature, has advanced to the stage where structures of many targets have or are being determined. These range in size and complexity, from small oligonucleotide fragments (Kennard & Hunter, 1991) to numerous enzymes and even to viruses such as polio and rhinovirus (Hogle, Chow & Filman, 1985; Rossmann & Johnson, 1989; Perutz, 1992). Some of these structures have proved useful in helping to identify drugs which have progressed through to clinical trials (Perutz, 1992; Bugg, *et al*. 1993; von Itzstein *et al*. 1993). If we are to make progress in the search for new antiparasite drugs we need input from pharmaceutical companies. Our basic research must be advertised as widely as possible and brought to their attention in order that symbiotic interactions might be promoted. It is the professional drug finders and designers (the pharmaceutical industry) who have the applied expertise to identify a molecule of therapeutic use and get it through to the afflicted. Given the vast stores of compounds and data already in place, who knows what antiparasite leads may already be stored in the drug company databanks?

We continue to learn and one lesson that has been reinforced is how complicated and difficult the drug discovery process can be. Nevertheless, there is cause for optimism combining a structure-based approach with the more traditional screening methods and it is to be hoped that it will not be long before more drugs are identified and society reaps rewards from this scientific endeavour.

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REFERENCES

ALLEN, F. H. & KENNARD, O. (1993). 3D search and research using the Cambridge Crystallographic Database. *Chemical Design Automation News* **8**, 130–137.

ATTWOOD, M. R., HASSELL, C. M., KROHN, A., LAWTON, G. & REDSHAW, S. (1986). The design and synthesis of the the angiotensin converting enzyme inhibitor Cilazapril and related bicyclic compounds. *Journal of the Chemical Society Perkin Transactions* **1,** 1011–1019.

BAILEY, S., SMITH, K., FAIRLAMB, A. H. AND HUNTER, W. N. (1993). Substrate interactions between trypanothione reductase and *N*1-glutathionylspermidine disulphide at 0·28 nm resolution. *European Journal of Biochemistry* **213**, 67–75.

BARRETT, M. P., PHILLIPS, C., ADAMS, M. J. & LE PAGE, R. W. (1994). Overexpression in *Escherichia coli* and purification of the 6-phosphogluconate dehydrogenase of *Trypanosoma brucei*. *Protein Expression and Purification* **5**, 44–49.

BELLO, A. R., NARE, B., FREEDMAN, D., HARDY, L. & BEVERLEY, S. M. (1994). PTR1: a reductase mediating salvage of oxidised pteridines and methotrexate resistance in the protozoan parasite *Leishmania major*. *Proceedings of the National Acadamy Sciences, USA*. **91**, 11442–11446.

BERNSTEIN, B. E., MICHELS, P. A. M. & HOL, W. G. J. (1997). Synergistic effects of substrate-induced conformational changes in phosphoglycerate kinase activation. *Nature* **385**, 275–278.

BILLSTEIN, S. A. (1994). How the pharmaceutical industry brings an antibiotic drug to market in the United States. *Antimicrobial Agents and Chemotherapy* **38**, 2679–2682.

BLACK, J. W. (1989). Drugs from emasculated hormones: the principle of syntopic antagonism. *Angewandte Chemie International Edition in English* **28**, 886–894.

BLUM, M. L., DOWN, J. A., GURNETT, A. M., CARRINGTON, M., TURNER, M. J. & WILEY, D. C. (1993). A structural motif in the variant surface glycoproteins of *Trypanosoma brucei*. *Nature* **362**, 603–609.

BLUNDELL, T. L. (1994). Metalloproteinase super-families and drug design. *Nature Structural Biology* **1**, 73–75.

BOLIN, J. T., FILMAN, D. J., MATHEWS, D. A., HAMLIN, R. C. & KRAUT, J. (1982). Crystal structure of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1·7 Å resolution: general features and binding of methotrexate. *Journal of Biological Chemistry* **257**, 13650–13658.

BORGES, A., CUNNINGHAM, M. L., TOVAR, J. & FAIRLAMB, A. H. (1995). Site-directed mutagenesis of the redox-active cysteines of *Trypanosoma cruzi* trypanothione reductase. *European Journal of Biochemistry* **228**, 745–752.

BORMAN, S. (1992). New 3-D search and *de novo* design techniques aid drug development. *Chemical & Engineering News* **10**, 18–26.

BOSHART, M. & MOTTRAM, J. C. (1997). Protein phosphorylation and protein kinases in trypanosomatids. In *Trypanosomaisis and Leishmaniasis : Biology and Control* (ed. Hide, G., Mottram, J. C., Coombs, G. H. & Holmes, P. H.), pp. 227–244. CAB International, Oxford, UK.

BUGG, C. E., CARSON, W. M. & MONTGOMERY, J. A. (1993). Drugs by design. *Scientific American* **12,** 60–66.

CHATTOPADHYAY, D., MOORE, D., CAMPBELL, P., BZIK, D., FOX, B. A., DELUCAS, L. J. & STHANA, V. L. (1996). Purification and crystallization of recombinant lactate dehydrogenase of *Plasmodium falciparum*. *International Union of Crystallography Seattle Congress Abstracts*. E1056.

COLE, P. A. (1996). Chaperone-assisted protein expression. *Structure* **4**, 239–242.

COLMAN, P. M. (1994). Structure-based drug design. *Current Opinion in Structural Biology* **4**, 868–874.

COLMAN, P. M., JANSONIUS, J. N. & MATTHEWS, B. W. (1972). The structure of thermolysin: an electron density map at 2·3 Å resolution. *Journal of Molecular Biology* **238**, 70–701.

CUNNINGHAM, M. L., ZVELEBIL, M. J. J. M. & FAIRLAMB, A. H. (1994). Mechanism of inhibition of trypanothione reductase by trivalent organic arsenicals. *European Journal of Biochemistry* **221**, 285–295.

DEGANO, M., GOPAUL, D. N. SCAPIN, G., SCHRAMM, V. L. & SACCHETTINI, J. C. (1996). Three-dimensional structure of the inosine-uridine nucleoside *N*-ribohydrolase from *Crithidia fasciculata*. *Biochemistry* **35**, 5971–5981.

DESJARLAIS, R. L., SEIBEL, G. L., KUNTZ, I. D., FURTH, P. S., ALVAREZ, J. C., ORTIZ DE MONTELLANO, P. R., DECAMP, D. L., BABE, L. M. & CRAIG, C. S. (1990). Structure-based design of non-peptide inhibitors specific for the human immunodeficiency virus 1 protease. *Proceedings of the National Acadamy of Sciences, USA* **87**, 6644–6648.

DUNN, C. R., BANFIELD, M. J., BARKER, J. J., HIGHAM, C. W., MORETON, K. M., TURGET-BALIK, D., BRADY, R. L. & HOLBROOK, J. J. (1996). The structure of lactate dehydrogenase from *Plasmodium falciparum* reveals a new target for anti-malarial design. *Nature Structural Biology* **3**, 912–915.

ELION, G. B. (1989). The purine path to chemotherapy. *Angewandte Chemie International Edition in English* **28**, 870–878.

FAIRLAMB, A. H. (1989). Novel biochemical pathways in parasitic protozoa. *Parasitology* **99S**, 93–112.

FAIRLAMB, A. H. AND CERAMI, A. (1992). Metabolism and functions of trypanothione in the Kinetoplastida. *Annual Reviews of Microbiology* **46**, 695–729.

FITZGERALD, P. M. D. (1994). HIV protease-ligand complexes. *Current Opinion in Structural Biology* **4**, 351–364.

FOCIA, P. J., FREYMANN, D. M., SOMOZA, J. R., WANG, C. C & FLETTERICK, R. J. (1996). The 1·8 Å resolution crystal structure of HGPRTASE from the human parasite *S. mansoni* with bound inhibitor. *International Union of Crystallography*, *Seattle*, *Congress Abstracts E*1251.

GRISHIN, N. V., OSTERMAN, A. L., GOLDSMITH, E. J. & PHILLIPS, M. A. (1996). Crystallisation and preliminary X-ray studies of ornithine decarboxylase from *Trypanosoma brucei*. *Proteins : Structure, Function and Genetics* **24**, 272–273.

GUTTERIDGE, W. E. (1993). Chemotherapy. In *Modern Parasitology* (ed Cox, F. E. G.), pp. 219–242. Blackwell Scientific Publications, Oxford UK.

HASSELL, C. M., KROHN, A., MOODY, C. H. & THOMAS, W. A. (1984). The design and synthesis of new triazolo- and pyridazo-pyridazin derivatives as inhibitors of angiotensin converting enzyme. *Journal of the Chemical Society Perkin Transactions* **1,** 155–164.

HITCHINGS, G. H. (1969). Chemotherapy and comparative biochemistry: G. M. A. Clowes memorial lecture. *Cancer Research* **29,** 1895–1900.

HITCHINGS, G. H. (1989). Selective inhibitors of dihydrofolate reductase. *Angewandte Chemie International Edition in English* **28**, 879–885.

HOGLE, J. M., CHOW, M. & FILMAN, D. J. (1985). Three-dimensional structure of poliovirus at 2·9 Å resolution. *Science* **229,** 1358–1365.

HOL, W. G. J. (1986). Protein crystallography and computer graphics-toward rational drug design. *Angewandte Chemie International Edition in English* **25**, 767–852.

HUANG, K., STRYNADKA, N. C., BERNARD, V. D., PEANASKY, R. J. & JAMES, M. N. G (1994). The molecular structure of the complex of *Ascaris* chymotrypsin/elastase inhibitor with porcine elastase. *Structure* **2**, 679–689.

HUNTER, W. N., BAILEY, S., HABASH, J., HARROP, S. J., HELLIWELL, J. R., ABOAGYE-KWARTENG, T., SMITH, K. & FAIRLAMB, A. H. (1992). Active site of trypanothione reductase a target for rational drug design. *Journal of Molecular Biology* **227**, 322–333.

HYDE, J. E. (1990). *Molecular Parasitology*. Open University Press, Milton Keynes, UK.

JOHNSON, L. N. (1994). Structure-based drug design. *International Union of Crystallography*, *Newsletter* **2**, 5–8.

KAUFMAN, H. E. (1993). The first effective antiviral. In *The Search for Antiviral Drugs ; Case Histories from Concept to Clinic* (ed. Adams, J. & Merluzzi, V. J.), pp. 1–24. Birkhauser, Boston, U.S.A.

KENNARD, O. AND HUNTER, W. N. (1991). Single-crystal X-ray diffraction studies of oligonucleotides and oligonucleotide-drug complexes. *Angewandte Chemie International Edition in English* **30**, 1254–1277.

KIM, H., FEIL, I. K., VERLINDE, C. L. M. J., PETRA, P. H. & HOL, W. G. J. (1995). Crystal structure of glycosomal glyceraldehyde-3-phosphate dehydrogenase from *Leishmania mexicana*: implications for structure-based drug design and a new position for the inorganic phosphate binding site. *Biochemistry* **34**, 14975–14986.

KNIGHTON, D. R., KAN, C. C, HOWLAND, E., JANSON, C. A., HOSTOMSKA, Z., WELSH, K. M. & MATTHEWS, D. A. (1994). Structure of and kinetic channelling in bifunctional dihydrofolate reductase-thymidylate synthase. *Nature Structure Biology* **1**, 186–194.

KOHLSTAEDT, L. A., WANG, J., FRIEDMAN, J. M., RICE, P. A. & STEITZ, T. A. (1992). Crystal structure at 3·5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* **256,** 1783–1790.

KRAULIS, P. J. (1991). MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *Journal of Applied Crystallography* **24**, 946–950.

KUNTZ, I. D., MENG, E. C. & SHOICHET, B. K. (1994). Structure-based molecular design. *Accounts of Chemical Research* **27**, 117–123.

KURIYAN, J., KONG, X.-P., KRISHNA, T. S. R., SWEET, R. M., MURGOLO, N. J., FIELD, H., CERAMI, A & HENDERSON, G. B. (1991). X-ray structure of trypanothione reductase from *Crithidia fasciculata* at 2·4 Å resolution. *Proceedings of the National Academy of Sciences, USA* **88**, 8764–8768.

LANTWIN, C. B., SCHLICHTING, I., KABSCH, W., PAI, E. F. & KRAUTH-SIEGEL, R. L. (1994). The structure of *Trypanosoma cruzi* trypanothione reductase in the oxidised and NADPH reduced state. *Proteins : Structure, Function and Genetics* **18**, 161–173.

LIM, K., HO, J. X., KEELING, K., GILLILAND, G. L., JI, X., RUKER, F. & CARTER, D. C. (1994). Three-dimensional structure of *Schistosoma japonicum* glutathione-S-transferase fused with a six-amino acid conserved neutralizing epitope of gp41 from HIV. *Protein Science* **3**, 2233–2237.

LIPSCOMB, W. N. (1970). Structure and mechanism in the enzymatic activity of carboxypeptidase A and relations to chemical sequence. *Accounts of Chemical Research* **3**, 81–89.

MANDELL, G. L. & SANDE, M. A. (1990). Antimicrobial agents. In *The pharmacological Basis of Therapeutics* (ed. Gilman, A. G., Rall, T. W., Nies, A. S. & Taylor, P.), pp. 1146–1164. Pergammon Press, New York, USA.

McGRATH, M. E., EAKIN, A. E., ENGEL, J. C., McKERROW, J. H., CRAIK, C. S. & FLETTERICK, R. J. (1995). The crystal structure of cruzain: a therapeutic target for Chagas disease. *Journal of Molecular Biology* **247**, 251–259.

McTIGUE, M. A., WILLIAMS, D. R. & TAINER, J. A. (1995). Crystal structures of a schistosomal drug and vaccine target: glutathione-S-transferase from *Schistosoma japonicum* and its complex with the leading antischistosomal drug praziquantel. *Journal of Molecular Biology* **246**, 21–27.

MITCHISON, T. J. (1994). Towards a pharmacological genetics. *Chemistry & Biology* **1**, 3–6.

MOTTRAM, J. C. & GRANT, K. M. (1996). *Leishmania mexicana* p12^{cks1}, a homologue of fission yeast p13^{suc1} associates with a stage-regulated histone H1 kinase. *Biochemical Journal* **316**, 833–839.

NIELSEN, J. (1994). Combinatorial chemistry. *Chemistry & Industry* **22**, 902–905.

NIES, A. S. (1990). Principles of therapeutics. In *The Pharmacological Basis of Therapeutics* (ed Gilman, A. G., Rall, T. W., Nies, A. S. & Taylor, P.), pp. 62–83. Pergamon Press New York, USA.

OLIVA, G., SOUZA, D. H. F., ARAUJO, A. P. U. & JESUS, W. D. P. (1996). The crystal structure of *T. cruzi* glycosomal glyceraldehyde-3-phosphate dehydrogenase: implications for the catalytic mechanism and new potential target sites for selective inhibition. *International Union of Crystallography, Seattle, Congress Abstracts* E0749.

ORENGO, C. A., JONES, D. T. & THORNTON, J. M. (1994). Protein superfamilies and domain superfolds. *Nature* **372,** 631–634.

O'BRIEN, C. (1994). HIV integrase structure catalyzes drug search. *Science* **266**, 1946.

PECK, A. W. (1994). Lamotrigine: historical background. *Reviews in Contemporary Pharmacotherapy* **5**, 95–105.

PERUTZ, M. (1992). *Protein Structure*. W. H. Freeman and Co. New York, USA.

PONASEK, J. A., STRICKLAND, C., FAERMAN, C., SAVVIDES, S., KARPLUS, P. A. & GANEM, B. (1995) Kukoamine A and other hydrophobic acylpolyamines: potent and selective inhibitors of *Crithidia fasciculata* trypanothione reductase. *Biochemical Journal* **311**, 371–375.

RING, C. S., SUN, E., MCKERROW, J. H., LEE, G. K., ROSENTHAL, P. J. KUNTZ, I. D. & COHEN, P. (1993). Structure based inhibitor design by using protein models for the development of antiparasitic agents. *Proceedings of the National Academy of Sciences, USA* **90**, 3583–3587.

ROSSMANN, M. G. & JOHNSON, J. E. (1989). Icosahedral RNA virus structure. *Annual Reviews in Biochemistry* **58**, 533–573.

SCHIRMER, R. H., MULLER, J. G. & KRAUTH-SIEGEL, R. L. (1995). Disulfide-reductase inhibitors as chemotherapeutic agents: the design of drugs for trypanosomiasis and malaria. *Angewandte Chemie International Edition in English* **34**, 141–154.

SCHLAGENHAUF, E., ETGES, R. & METCALF, P. (1995). Crystallisation and preliminary X-ray diffraction studies of Leishmanolysin, the major surface metalloproteinase from *Leishmania major*. *Proteins : Structure, Function and Genetics* **22**, 58–66.

SHOICHET, B. K., STROUD, R. M., SANTI, D. V., KUNTZ, I. D. & PERRY, K. M. (1993). Structure-based discovery of inhibitors of thymidylate synthase. *Science* **259**, 1445–1450.

SOMOZA, J. R., CHIN, M. S., FOCIA, P. J., WANG, C. C. & FLETTERICK, R. J. (1996). Crystal structure of the hypoxanthine-guanine-xanthine phosphoribosyltransferase from the protozoan parasite *Tritrichomonas foetus*. *Biochemistry* **35**, 7032–7040.

TRAVIS, A. S. (1991). Paul Ehrlich: 100 years of chemotherapy 1891–1991. *The Biochemist* **13,** 9–12

TROTTEIN, F., VANEY, M. C., BACHET, B., PIERCE, R. J., COLLOC'H, N., LECOCQ, J. P., CAPRON, A. & MONNON, J. P. (1992). Crystallisation and preliminary X-ray diffraction studies of a proctective 28kDa glutathione-S-transferase from *Schistosoma mansoni*. *Journal of Molecular Biology* **224**, 515–518.

VAGELOS, P. R. (1991). Are prescription drug prices high? *Science* **252**, 1080–1084.

VAN CALENBERGH, S., VERLINDE, C. L., SOENENS, J., DE BRUYN, A., CALLENS, M., BLATON, N. M., PEETERS, O. M., ROZENSKI, J., HOL, W. G. & HERDEWIJN, P. (1955). Synthesis and structure-activity relationships of analogs of 2′deoxy-2′(3-methoxybenzamido)adenosine; a selective inhibitor of trypanosomal glycosomal glyceraldehyde-3-phosphate dehydrogenase. *Journal of Medicinal Chemistry* **38**, 3838–3849.

VARGHESE, J. N. AND COLMAN, P. M. (1991). Three-dimensional structure of the neuraminidase of influenza virus A/Tokyo/3/67 at 2·7 Å resolution. *Journal of Molecular Biology* **221**, 473–480.

VERLINDE, C. L. M. J. & HOL, W. G. J. (1994). Structure-based drug design: progress, results and challenges. *Structure* **7**, 577–587.

https://doi.org/10.1017/S0031182097008962 Published online by Cambridge University Press

VERLINDE, C. L., WITMANS, C. J., PIJNING, T., KALK, K. H., HOL, W. G., CALLENS, M. & OPPERDOES, F. R. (1992). Structure of the complex betwen trypanosomal triosephosphate isomerase and *N*-hydroxy-4-phosphono-butanamide: binding at the active site despite an 'open' flexible loop conformation. *Protein Science* **1**, 1578–1584.

VON ITZSTEIN, M., WU, W.-Y., KOK, G. B., PEGG, M. S., DYASON, J. C., JIN, B., PHAN, T. V., SYMTHE, M. L., WHITE, H. F., OLIVER, S. W. & PENN, C. R. (1993). Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* **363**, 418–423.

WERTH, B. (1994). *The Billion Dollar Molecule, One Company's Quest for the Perfect Drug*. Simon and Schuster, New York, USA.

WHITBY, F. G., HUETE-PEREZ, J., LUECKE, H. & WANG, C. C. (1995). Preliminary X-ray crystallographic analysis of *Tritrichomonas foetus* inosine-5′-monophosphate dehydrogenase. *Proteins : Structure, Function and Genetics* **23**, 598–603.

WIERENGA, R. K., KALK, K. H. & HOL, W. G. (1987). Structure determination of the glycosomal trisoephosphate isomerase from *Trypanosoma brucei brucei* at 2·4 Å resolution. *Journal of Molecular Biology* **198**, 109–121.

WONG, C.-H. & WHITESIDES, G. M. (1994). Enzymes in synthetic organic chemistry. *Tetrahedron Organic Chemistry Series*, Volume **12**. Pergamon Press/Elsevier Science Ltd, Oxford, UK.

WUTHRICH, K. (1995). NMR – This other method for protein and nucleic acid structure determination. *Acta Crystallographica* **D51**, 249–270.

YANG, J., KLOEK, A. P., GOLDBERG, D. E. & MATHEWS, F. S. (1995). The structure of *Ascaris* haemoglobin domain I at 2·2 Å resolution: molecular features of oxygen avidity. *Proceedings of the National Academy of Sciences, USA* **92**, 4224–4228.

ZHANG, Y., BOND, C. S., BAILEY, S., CUNNINGHAM, M. L., FAIRLAMB, A. H. & HUNTER, W. N. (1996). The crystal structure of trypanothione reductase from the human pathogen *Trypanosoma cruzi* at 2·3 Å resolution. *Protein Science* **5**, 52–61.