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Deformylase as a novel antibacterial target

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Bacterial genomics has revealed a plethora of previously unknown targets of potential use in the discovery of novel antibacterial drugs. However, so far little has emerged from this approach. Peptide deformylase is an interesting target that was discovered more than 30 years ago, but was not exploited until recently. The reawakening of interest in this target resulted from an improved understanding of the enzyme, making it a more tractable and attractive target. Information on the properties of the enzyme, such as its three-dimensional structure, the activity of inhibitors, its resistance and suitability as a target are discussed.

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▼ The discovery of antibiotics and their impact on bacterial infections represents one of the great success stories of 20th-century medicine but there is a growing concern about increasing antibiotic resistance. Ultimately, bacteria have proven to be capable of developing resistance to any antibiotic that has been used clinically, the only unknown being when, not if! The ability of antibiotics to safely cure infections depends on their exploitation of the differences between the biochemical pathways in bacteria and human cells. Targets for antibacterial drug discovery can be chosen rationally on the basis of such differences. Although there is a large number of antibiotics used clinically, the variety of targets that they inhibit is limited. The analysis of microbial genomes has revealed an abundance of novel and potentially useful targets[1] but, so far, little has resulted from this much heralded effort. One target that had not received much attention until recently is peptide deformylase (PDF; EC 3.5.1.31).

Function of PDF

Protein synthesis is a proven rich source of targets for antibacterial drugs[2]. Many of the known antibiotics (e.g. the aminoglycosides, macrolides, tetracyclines and oxazolidinones) work by inhibiting one or more steps of this complex process. Although the protein synthesizing machinery of bacterial and mammalian cells is similar overall, there is enough of a difference to allow for the selective blocking of this process in bacteria. One significant difference is the transformylation and subsequent deformylation of methionine, as shown in Fig. 1 (Refs 3,4). Unlike cytosolic protein synthesis in mammalian cells, protein synthesis in bacteria and mitochondria is initiated by *N*-formylmethionine[5,6], which is formed from methionyl-tRNA by *N*-methionyl-tRNA transformylase. In prokaryotes, but not in mitochondria, the *N*-formylmethionine of the nascent protein is removed sequentially by the action of PDF and a methionine amino peptidase to produce the mature protein[5,7]. This role of PDF in bacterial protein synthesis provides a rational basis for selectivity, making it an attractive target for drug discovery. Recently, the possible use of PDF as an antimicrobial target has been reviewed by Giglione and coworkers[8].

The essential role of deformylase in bacteria

Currently used antibiotics work by inhibiting the growth of, or killing, bacteria. In the continuing search for new antibiotics that work in this way, it is crucial that any new chosen target is essential for the growth and/or survival of the bacteria. Although alternative approaches to therapy, such as inhibition of virulence, have been attempted, no new drugs have yet emerged from such efforts. The essential nature of deformylase was first suggested in 1994 by two groups[4,9] working with *Escherichia coli*. In one case, it was shown that the deformylase gene (*def*) could not be inactivated unless the transformylase gene (*fmt*) was also inactivated[4]. In the other case,

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a conditional-lethal (temperature-sensitive) mutant was constructed[9]. More recently, the same conclusions have been reached with *Staphylococcus aureus*[10] using the first approach. The inactivation of *def* cannot be achieved in *Streptococcus pneumoniae*[11], which suggests essentiality. Recently, more conclusive evidence concerning the essentiality of *def* in *E. coli* was provided by the construction of an arabinose-dependent strain that has a chromosomal copy of *def* under the control of the arabinose-inducible promoter P_{BAD} (Ref. 10). Growth depended on the presence of the inducer, arabinose. The *fmt* gene itself is not essential in *E. coli*[4], *S. aureus*[10] or *Pseudomonas aeruginosa*[12]. Mutants in *fmt* have been described in these species and all have an impaired growth phenotype. By contrast, it appears that *fmt* is essential in *S. pneumoniae*[11] because it has not proved possible to inactivate it.

Obviously, in the absence of transformylase activity there is no need to deformylate a nascent peptide. However, it does raise the question as to why bacteria add a formyl group, and subsequently remove it, if it is not necessary in the first place. The slowed growth defect of *fmt* mutants is most severe in *E. coli* and least severe in *P. aeruginosa*. Thus there is a varying price to be paid for elimination of the formylation–deformylation cycle.

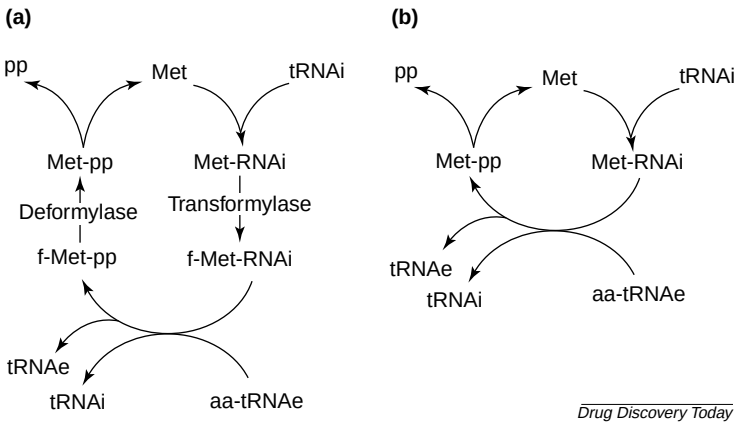

Figure 1. Initiation of protein synthesis by methionine in **(a)** bacterial and **(b)** mammalian cells. Protein synthesis in mammalian and bacterial cells always begins with the amino acid methionine. This initiating amino acid is subsequently removed by the enzyme, methionine amino peptidase. Although the overall process is similar, there is a significant difference: bacteria first formylate and then subsequently deformylate the initiating methionine. If the formyl group is not removed (by the enzyme deformylase), protein synthesis is inhibited and the bacteria cannot grow. Abbreviations: Met, methionine; f-Met, formylmethionine; pp, polypeptide; aa, amino acid; tRNAi, initiatior transfer RNA; tRNAe, elongation transfer RNA.

Is deformylase present in all bacteria?

The distribution of PDF gene homologs has been studied extensively. Orthologs of deformylase (homolog genes that code for proteins of the same function) are present in all bacterial genomes that have been sequenced completely, and most incomplete genomes have contigs with a PDF homolog (see Fig. 2; Refs 10,13). The wide distribution of PDF homologs in bacteria suggests that it could provide the basis for discovering broad-spectrum antibacterial drugs. Genomic analysis shows that bacteria have at least one homolog, sometimes two. Often, PDF homologs are in a dicistronic operon with transformylase; in other bacteria the PDF gene is elsewhere[3,10]. All Gram-positive bacteria that have been examined, and a few Gram-negative bacteria, have two homologs. In *S. aureus*[10] and *S. pneumoniae*[11] only one copy, *defB*, has the conserved domains associated with the active site whereas the other copy, *defA*, does not. This second copy is frequently not a functional PDF; it is a deformylase paralog – a homolog gene that codes for a protein with a different function – with an unknown function. Genetic analysis in *S. aureus* and *S. pneumoniae* has shown that *defB* cannot be disrupted in the wild-type strain, whereas the *defA* paralog can be disrupted under laboratory conditions[10]. This suggests that *defB* is an essential gene that encodes the active deformylase.

The presence and relevance of deformylase in eukaryotes

Several eukaryotic parasites (e.g. *Plasmodium falciparum* and trypanosomal species) and plants[14] have a PDF gene homolog. Interestingly, the presence of PDF in lower eukaryotes yields potential applications of PDF inhibitors in important diseases, such as malaria or Chaga's disease[14]. The presence of PDF activity in humans has been reported in relation to the degradation of formyl-peptides of bacterial origin in the mucosal barrier of the gut[15] but this was apparently only active on formylmethionine and not formylated peptides. Although proteins that are synthesized in the mitochondria can be formylated, they do not appear to have their formyl moiety removed. Recently, a human expressed sequence tag (EST) was identified that contained a homolog of PDF, and the corresponding cDNA was cloned[16]. The significance of this finding is not clear but the deduced sequence of this PDF most closely resembles that of a mitochondrial PDF from plant and insect sources. PDF might have a role in the synthesis of mitochondrial proteins in animal and human cells that is yet to be established. Even if this proves to be true, it does not invalidate the use of PDF as a bacterial target. Currently,

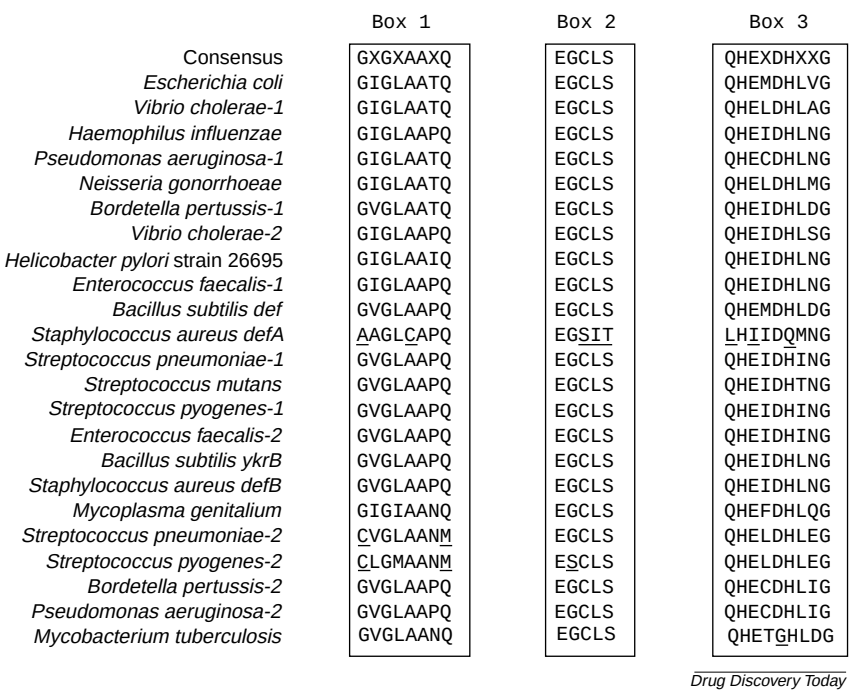

Figure 2. Alignment of predicted catalytic domains of deformylase. The availability of genome sequences for many bacteria has permitted the identification and comparison of the deformylase gene homologs present. Certain portions of the deformylase gene sequence are highly conserved and correspond to the catalytic domains of the enzyme. The three conserved motifs that define the catalytic domain of deformylase are shown. Position of the motifs in the *Esherichia coli* protein; Box 1, G44 to Q51; Box 2, E89 to S93; Box 3, Q132 to G140. The few residues that diverge from the consensus are underlined. In some cases bacteria have two deformylase genes, both or one of which might code for a functional enzyme. In the case of *Staphylococcus aureus defA,* it can be seen that there are a total of eight changes within the three consensus sequences, not surprisingly it does not code for a functional deformylase.

several approved antibiotics are known to inhibit protein synthesis in mitochondria (e.g. macrolides, tetracyclines and chloramphenicol[17]). Alternatively, the human PDF homolog could be an ancestral remnant with no function.

Why has so little progress been made with deformylase until recently?

In the early 1960s, it was established that formylmethionyl-tRNA initiates bacterial protein synthesis[5]. However, because few (if any) mature proteins in *E. coli* retain formylmethionine, it was suspected that a specific enzyme was responsible for removal of the formyl group and PDF activity was first reported by Adams in 1968 (Ref. 18). This study showed that a protein fraction from *E. coli* was capable of removing the formyl group from the nascent protein. However, subsequent attempts to purify the activity of PDF failed because it was not stable[18]. The inability to purify this enzyme prevented further characterization until the cloning of a deformylase gene, *def*, in 1993 (Ref. 19). The *def* gene was subsequently overexpressed in *E. coli* and the protein product purified to homogeneity by gel filtration, anion exchange and hydrophobic interaction chromatographies[19,20]. The purified protein had low deformylase activity ($k_{cat}/K_m = 80$ M^{-1} sec^{-1} with formyl-Met–Ala–Ser as substrate) and was sensitive to metal chelators such as 1,10-phenanthroline and EDTA. Examination of the predicted sequences of the *E. coli* PDF revealed the presence of a typical zinc-binding motif, HEXXH (where X denotes any amino acid), which is characteristic of the metallopeptidases. Consistent with this observation, the purified protein contains approximately one zinc ion per polypeptide. Based on these data, it was concluded that PDF belongs to the zinc-hydrolase superfamily[20].

In 1997, Rajagopalan *et al.*[21] reported that a protein fraction with high PDF activity ($k_{cat}/K_m = 2.9 \times 10^4$ M^{-1} sec^{-1} with formyl-Met–Ala–Ser as substrate) could be purified under oxygen-free conditions from *E. coli* overexpressing PDF. This highly active PDF contains one ferrous ion per polypeptide and was proposed to be the physiological form of the enzyme. The poorly active Zn^{2+}-containing PDF that had been purified previously, was postulated to be an artifact of overexpression. The ferrous-containing PDF had actually been described in 1995 (D. Groche, PhD thesis, Universität Heidelberg, Germany) but the work was not published until 1998 (Ref. 22). The ferrous-containing PDF is not stable and can quickly lose its enzymatic activity as a result of the oxidation of ferrous ion to ferric ion, through contact with atmospheric oxygen[23]. The instability of ferrous-ion-containing PDF explains the failure to purify active PDF enzyme during the past 30 years. The finding that nickel can replace the ferrous ion to produce a highly active and stable enzyme was a key discovery[22,24], because the extreme lability of the physiological form made its use in HTS impractical. The ferrous ion of native PDF from overproducing bacteria can be exchanged directly with a nickel ion in the presence of an excess of nickel ion. This nickel-containing PDF is oxygen insensitive and catalytically as active as the native ferrous-containing PDF (Refs 22,25). Alternatively, cobalt-containing PDF can be used as an

active surrogate[26]. The nickel- and cobalt-containing PDFs are stable under typical assay conditions and provide a reliable form of enzyme for biochemical screening and studying SARs.

These surrogate PDFs paved the way for subsequent research and were used in efforts to discover PDF inhibitors with antibacterial activity[27–35]. The first report regarding the use of PDF as a potential antibacterial drug-discovery target came in 1998 (Ref. 36).

Discovering inhibitors of PDF

The discovery of novel antibiotics has been largely an empirical screening process; typically, antibacterial activity is discovered first and the structure of the molecule responsible and the targets involved are established afterwards. Structure- and mechanism-based drug designs represent rational approaches. The fact that PDF is a metalloprotease gives it an added attractiveness as a target for drug discovery. Metalloproteases are among the best studied of enzyme classes[37] and there are excellent precedents for the mechanism-based design of their inhibitors. Perhaps the best known metalloprotease inhibitors are those of the angiotensin-converting enzyme (ACE; Ref. 38), which have generated billions of dollars in sales for the pharmaceutical industry as antihypertensive agents. The commercial interest in ACE inhibitors has helped propel the general understanding of metalloproteases. Recently, the interest of the pharmaceutical industry has focused on matrix metalloproteases (MMP; Ref. 39), which has further advanced knowledge of how to design the corresponding inhibitors, in most cases as potential anticancer agents.

The three-dimensional (3D) structures of various PDFs have been solved and published, including structures of enzyme–inhibitor complexes[22,34,40–45]. The 3D structures obtained previously were based on the zinc-containing enzyme but are similar to the structures of more recent reports using the nickel- or ferrous-containing enzyme[40]. It was noted that, although it is a ferrous aminopeptidase with a primary sequence different from other metalloproteases, the environment of the catalytic metal ion in PDF appears similar to the active sites of thermolysin and the MMPs (Ref. 42). In the published structure, the metal ion at the active site of the *E. coli* PDF is surrounded tetrahedrally by the side chains of Cys90, His132, His136 and a water molecule. In PDF structures with bound inhibitor, a chelating group replaces this water molecule. In all of the structures, the metal-binding site is adjacent to a deep hydrophobic pocket, which accommodates the methionine side-chain. The availability of 3D PDF structural information and its similarity to that of the MMPs (Refs 24,41,42) makes it possible to perform *de novo* structure-based design

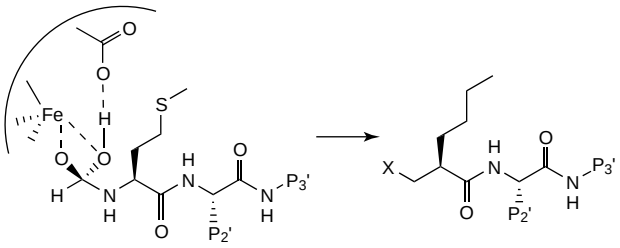

Figure 3. A generic PDF inhibitor structure. The transition state structure corresponds to a formylmethionyl peptide bound to a ferrous ion at the active site of the deformylase enzyme. In the generic inhibitor shown, X corresponds to a pharmacophore that can interact with the metal ion. The pharmacophore is attached to a molecule mimicking the methionyl peptide moiety of the normal substrate.

of PDF inhibitors, in addition to the mechanism-based design approach already mentioned. Based on the mechanistic and structural information, together with an understanding of the general principles of inhibiting metalloproteases, a generic inhibitor structure has been proposed (Fig. 3).

In the past few years, several classes of PDF inhibitors have been reported; their structures and activities are summarized in Fig. 4 and Table 1. Of these different classes of PDF inhibitors, only biaryl acid and the recently published thyropropic-acid derivatives[35] do not fit the generic inhibitor structure shown in Fig. 3. Although all these compounds inhibit PDF activity, most of them do not have antibacterial activity, presumably because of the weak potency against PDF and/or the inability to penetrate the bacterial cell. It is interesting to note that all the compounds with IC_{50} (or K_i) values >1 μM had no antibacterial activity. By contrast, the three classes of inhibitors that do have antibacterial activity all contain chelating groups (i.e. hydroxamate, *N*-formyl hydroxyl, or thiol). Also shown in Fig. 4 is actinonin, a naturally occurring antibiotic that was first isolated in 1962 from an actinomycete[46] and was recently shown to be a PDF inhibitor[28].

Is antibacterial activity a result of deformylase inhibition?

The fact that a potent PDF inhibitor also inhibits the growth of an intact bacterial cell does not prove that PDF inhibition causes the growth defect. Evidence that the same mechanism of action is involved has come from several lines of research. One of these methods[28] involves the use of the aforementioned *E. coli* genetic construct in

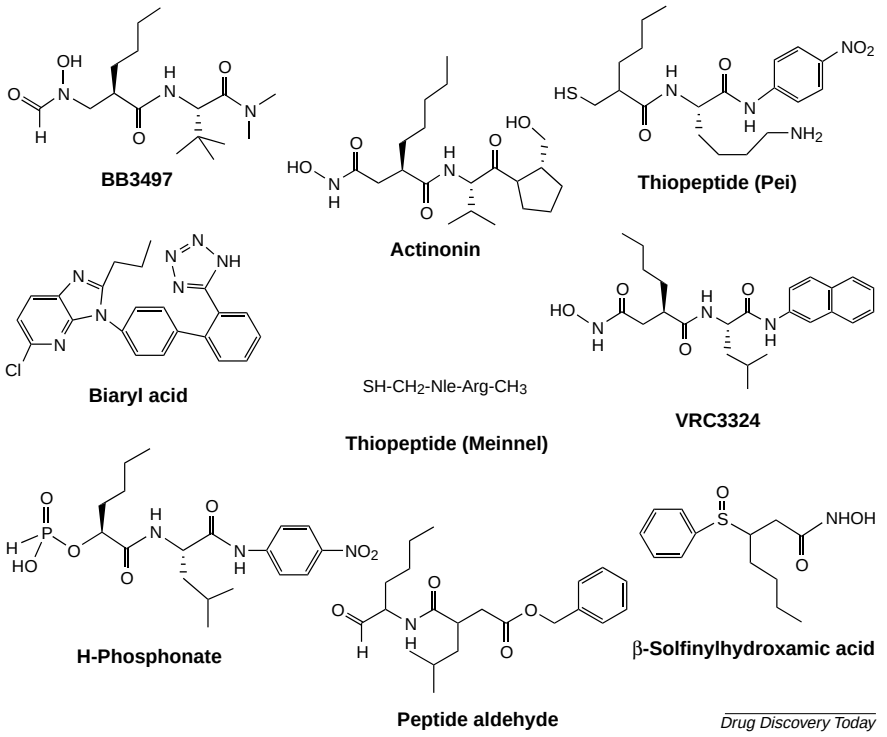

Figure 4. Structures of published PDF inhibitors. The variety of PDF inhibitor structures that have been described in the literature is shown here. For references, see Table 1.

Table 1. A summary of published peptide deformylase (PDF) inhibitors

Name of inhibitor	Ref.	IC_{50} (μM)	MIC (μg ml^{-1})
BB3497	33	0.007 (Nickel)	4–16 (*S. aureus*) 4 (*S. pneumoniae*)
Actinonin	28	0.0003 (K_i) (Nickel)	8–16 (*S. aureus*) 8 (*S. pneumoniae*)
Biaryl acid	49	3.9 (Cobalt)	NA
Peptide aldehyde	50	10.2 (Cobalt)	NA
H-phosphonate	36	37 (K_i) (Ferrous)	NA
Thiopeptide (Meinnel)	29	2.5 (K_i) (Nickel)	NA
Thiopeptide (Pei)	30	0.019 (K_i) (Nickel)	30–40 (*E. coli*) 2.6 (*B. subtilis*)
β-Solfinylhydroxamic acid	31	0.1 (Nickel)	16 (*S. aureus*) 32 (*S. pneumoniae*)
VRC3324	45	0.014 (Nickel)	1–2 (*S. aureus*) 1 (*S. pneumoniae*)

Abbreviation: MIC, minimum inhibitory concentration; NA, not applicable; *S. aureus*, *Staphylococcus aureas*; *S. pneumoniae*, *Streptococcus pneumoniae*; *E. coli*, *Escherichia coli*; *B. subtilis*, *Bacillus subtilis*.

which the sole chromosomal copy of the *def* gene is under the control of the arabinose-regulated P_{BAD} promoter. In this case, it was shown that the susceptibility to PDF inhibitors is associated with the concentration of the inducer (arabinose) in the growth medium, which regulates the amount of PDF inside the bacterial cell. This association is absent when testing antibiotics that inhibit other targets (Fig. 5). Using another approach, it has been shown that inactivation of the *fmt* gene[10,34,47] in *S. aureus* and *E. coli* results in resistance to PDF inhibitors. These mutants lack active transformylase, are growth impaired and are resistant to all antibacterial compounds that exclusively inhibit deformylase. A similar effect can be achieved by growing *E. coli* in rich media in the presence of trimethoprim and thymine[47]. Trimethoprim inhibits dihydrofolate reductase, which depletes the tetrahydrofolate pool. This mimics the *fmt* mutation because the lack of available folate forces protein synthesis to initiate with methionine that has not been formylated[28].

Resistance to deformylase inhibitors

The frequency and mechanism of resistance to PDF inhibitors have been explored in *S. aureus*, *E. coli*, *H. influenzae* and *S. pneumoniae*[10,11,34,47]. In *S. aureus* and *H. influenzae*, spontaneous mutants occur at frequencies of 1×10^{-6} and 1×10^{-8}, respectively. In both organisms the mutants harbored changes in the transformylase gene. It was already known that transformylase is not essential in *E. coli* and can provide a genetic background in which deformylase activity is no longer required for growth, so it was not surprising that PDF inhibitors select resistant mutants with changes in *fmt*. Expression of wild-type transformylase in *trans*, can restore the

susceptibility of a mutant resistant to PDF inhibitors in *S. aureus*[10]. As expected, deletion of the *fmt* gene in *S. aureus* also leads to resistance. Transformylase mutants in *S. aureus*, *H. influenzae*, *E. coli* or *P. aeruginosa* all have a slow growth phenotype, which suggests that protein synthesis proceeds less efficiently in the absence of the formylation–deformylation cycle. The effect of this growth impairment on pathogenicity was tested by using an *S. aureus* PDF inhibitor-resistant strain in a murine thigh-abscess model. Results showed that resistant mutants were significantly attenuated compared with the isogenic wild-type bacteria from which they were derived[10]. This diminished virulence (caused by transformylase inactivation) could preclude this type of resistance from being selected during therapy. In the case of *S. pneumoniae*, the frequency of resistant mutants is 1×10^{-8}. As mentioned previously, the *fmt* gene in this species appears to be essential. In keeping with this finding is the observation that resistance to PDF inhibitors is a result of mutations in *defB* and not in *fmt* (Ref. 11).

Activity of deformylase inhibitors *in vivo*

Although the natural product actinonin is a PDF inhibitor and has *in vitro* activity against bacteria, it is inactive *in vivo*[48]. The validation of PDF as an *in vivo* target was provided recently by two synthetic PDF inhibitors VRC3375 (Ref. 27) and BB3497 (Ref. 34) through the curing of staphylococcal septicemia in mice. In both cases, treatments were effective following administration of the PDF inhibitors by the intravenous, subcutaneous or oral routes.

VRC3375 is a potent PDF inhibitor (K_i = 0.24 nM against Ni-PDF from *E. coli*) with a minimum inhibitory content (MIC) of 1 μg ml^{-1} against *S. aureus*. In a mouse model of staphylococcal septicemia this compound gave ED_{50} values (the dose at which 50% of animals survive the infection) of 32, 17 and 21 mg kg^{-1} by the intravenous, subcutaneous and oral routes of administration, respectively. When dosed orally at 100 mg kg^{-1} in mice, VRC3375 was rapidly absorbed and reached a maximum concentration of 45 μg^{-1} ml in the serum at 15 minutes. The drug was detected at concentrations >100 μg ml^{-1} in heart, kidney, lung and muscle tissue three minutes after injection. In an acute toxicity study in mice, the single dose LD_{50} values for VRC3375 were 447, >500 and >500 mg kg^{-1} by intravenous, subcutaneous and oral routes of administration[27] (D. Chen *et al.*, unpublished). This gives an LD_{50}:ED_{50} ratio of >20 by the oral route, and although this is only a crude estimate of the therapeutic index, it is encouraging.

In a separate study, BB3497, another potent PDF inhibitor (IC_{50} = 7 nM against Ni-PDF from *E. coli*) with an MIC of 4 μg ml^{-1} against *S. aureus*, exhibited good efficacy in a similar staphylococcal septicemia model, giving ED_{50} values of 7 and 8 mg kg^{-1} by intravenous and oral routes of administration. BB3497 was also rapidly absorbed following oral administration to rats at a dose of 100 mg kg^{-1}, reaching a maximum concentration of 24 μg ml^{-1} in the serum. By contrast, at 50 mg kg^{-1} no actinonin can be detected in the blood, explaining why it had failed to show activity in earlier experiments[34].

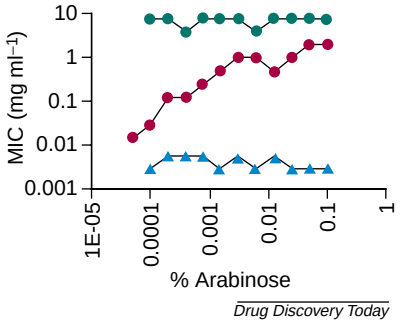

Figure 5. Actinonin inhibits bacterial growth through the inhibition of deformylase. Actinonin is a potent inhibitor of the deformylase enzyme and bacterial growth. To prove that deformylase inhibition was responsible for inhibition of bacterial growth, the susceptibility of an *E. coli* genetic construct with *def* under the control of the arabinose promoter was used. In this construct, altering the concentration of arabinose in the medium can regulate the concentration of deformylase inside a cell. At high concentrations of arabinose, the bacteria become resistant to actinonin (red circles) and at low concentrations they become more sensitive. Because high and low inducer concentrations correspond to high and low deformylase inside cells, this shows that it is inhibition of deformylase that inhibits bacterial growth. Other antibiotics that inhibit growth through different mechanisms not involving deformylase do not show any significant change in their effects at different arabinose concentrations: for example, fosfomycin (green circles) and ciprofloxacin (blue triangles). Reproduced, with permission, from Ref. 28 (copyright American Chemical Society).

Conclusions and future directions

In choosing an ideal target for the discovery of drugs that directly kill or inhibit the growth of bacteria, there are several important criteria to be considered. Bacterial PDF satisfies the majority of these: it is an essential enzyme, it is present in all clinically relevant bacteria and it is highly conserved. Although PDF has no role in mammalian cytoplasmic-protein synthesis, the recent identification of a human *def* gene homolog requires further study to better understand the potential impact of this homolog on the selectivity of PDF inhibitors. Information concerning the biochemical mechanism and structure of bacterial PDF is available and will be of great value in drug design. Information regarding the safety of PDF inhibitors is

awaited with interest and will require multiple dose toxicity studies in animals. The recent successful treatment of bacterial infections in an animal model using PDF inhibitors validates the use of this enzyme as a novel drug target. Based on the properties of the lead compounds, mainly their oral bioavailability, low frequency of resistance and good activity against common upper-respiratory pathogens (e.g. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*), one can envisage the development of a new antibiotic for community use. The use of PDF inhibitors as effective agents in the hospital setting remains open. The most frequent Gram-positive nosocomial species, *S. aureus*, shows a high frequency of resistance to this class of compounds, although the resistant mutants have attenuated virulence and might not emerge in a clinical setting.

The successful development of PDF inhibitors would add a new class of antibacterials to the shrinking arsenal of antibiotics available for the treatment of infections by resistant organisms.

References

1 Hutchison, C.A. *et al.* (1999) Global transposon mutagenesis and a minimal *Mycoplasma* genome. *Science* 286, 2165–2169
2 Leviton, I. (1999) Inhibitors of protein synthesis. *Cancer Invest.* 17, 87–92
3 Mazel, D. *et al.* (1997) A survey of polypeptide deformylase function throughout the eubacterial lineage. *J. Mol. Biol.* 266, 939–949
4 Mazel, D. *et al.* (1994) Genetic characterization of polypeptide deformylase, a distinctive enzyme of eubacterial translation. *EMBO J.* 13, 914–923
5 Adams, J.M. and Capecchi, M.R. (1966) N-formylmethionyl-sRNA as the initiator of protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 55, 147–155
6 Lucchini, G. and Bianchetti, R. (1980) Initiation of protein synthesis in isolated mitochondria and chloroplasts. *Biochim. Biophys. Acta* 608, 54–61
7 Meinnel, T. *et al.* (1993) Methionine as translation start signal: a review of the enzymes of the pathway in *Escherichia coli*. *Biochimie* 75, 1061–1075
8 Giglione, C. *et al.* (2000) Peptide deformylase as a target for new generation, broad spectrum antimicrobial agents. *Mol. Microbiol.* 36, 1197–1205
9 Meinnel, T. and Blanquet, S. (1994) Characterization of the *Thermus thermophilus* locus encoding peptide deformylase and methionyl-tRNA(fMet) formyltransferase. *J. Bacteriol.* 176, 7387–7390
10 Margolis, P.S. *et al.* (2000) Peptide deformylase in *Staphylococcus aureus*: resistance to inhibition is mediated by mutations in the formyltransferase gene. *Antimicrob. Agents Chemother.* 44, 1825–1831
11 Margolis, P.S. *et al.* (2001) Resistance of *Streptococcus pneumoniae* to deformylase inhibitors is due to mutations in defB. *Antimicrob. Agents Chemother.* 45, 2432–2435
12 Newton, D.T. *et al.* (1999) Formylation is not essential for initiation of protein synthesis in all eubacteria. *J. Biol. Chem.* 274, 22143–22146
13 Meinnel, T. *et al.* (1997) Structure–function relationships within the peptide deformylase family. Evidence for a conserved architecture of the active site involving three conserved motifs and a metal ion. *J. Mol. Biol.* 267, 749–761
14 Meinnel, T. (2000) Peptide deformylase of eukaryotic protists: a target for new antiparasitic agents? *Parasitol. Today* 16, 165–168
15 Chadwick, V.S. *et al.* (1990) Enzymes degrading bacterial chemotactic F-met peptides in human ileal and colonic mucosa. *J. Gastroenterol. Hepatol.* 5, 375–381
16 Giglione, C. *et al.* (2000) Identification of eukaryotic peptide deformylases reveals universality of N-terminal protein processing mechanisms. *EMBO J.* 19, 5916–5929
17 Anandatheerthavarada, H.K. *et al.* (1999) Physiological role of the N-terminal processed P4501A1 targeted to mitochondria in erythromycin metabolism and reversal of erythromycin-mediated inhibition of mitochondrial protein synthesis. *J. Biol. Chem.* 274, 6617–6625
18 Adams, J.M. (1968) On the release of the formyl group from nascent protein. *J. Mol. Biol.* 33, 571–589
19 Meinnel, T. and Blanquet, S. (1993) Evidence that peptide deformylase and methionyl-tRNA(fMet) formyltransferase are encoded within the same operon in *Escherichia coli*. *J. Bacteriol.* 175, 7737–7740
20 Meinnel, T. and Blanquet, S. (1995) Enzymatic properties of *Escherichia coli* peptide deformylase. *J. Bacteriol.* 177, 1883–1887
21 Rajagopalan, P.T.R. *et al.* (1997) Peptide deformylase, a new type of mononuclear iron protein. *J. Am. Chem. Soc.* 119, 12418–12419
22 Groche, D. *et al.* (1998) Isolation and crystallization of functionally competent *Escherichia coli* peptide deformylase forms containing either iron or nickel in the active site. *Biochem. Biophys. Res. Commun.* 246, 342–346
23 Rajagopalan, P.T. and Pei, D. (1998) Oxygen-mediated inactivation of peptide deformylase. *J. Biol. Chem.* 273, 22305–22310
24 Becker, A. *et al.* (1998) Iron center, substrate recognition and mechanism of peptide deformylase. *Nat. Struct. Biol.* 5, 1053–1058
25 Ragusa, S. *et al.* (1999) Substrate recognition and selectivity of peptide deformylase. Similarities and differences with metzincins and thermolysin. *J. Mol. Biol.* 289, 1445–1457
26 Rajagopalan, P.T. *et al.* (2000) Characterization of cobalt(II)-substituted peptide deformylase: function of the metal ion and the catalytic residue Glu-133. *Biochemistry* 39, 779–790
27 Chen, D. *et al.* (2000) *In vivo* evaluation of VRC3375, a potent peptide deformylase inhibitor. *40th Annual ICAAC Meeting*, Toronto, Canada (Abstract No. 2175)
28 Chen, D.Z. *et al.* (2000) Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. *Biochemistry* 39, 1256–1262
29 Meinnel, T. *et al.* (1999) Design and synthesis of substrate analogue inhibitors of peptide deformylase. *Biochemistry* 38, 4287–4295
30 Huntington, K.M. *et al.* (2000) Synthesis and antibacterial activity of peptide deformylase inhibitors. *Biochemistry* 39, 4543–4551
31 Apfel, C. *et al.* (2000) Hydroxamic acid derivatives as potent peptide deformylase inhibitors and antibacterial agents. *J. Med. Chem.* 43, 2324–2331
32 Clements, J.M. *et al.* (2000) *In vitro* and *in vivo* antibacterial activity of the peptide deformylase inhibitor BB3497. *40th Annual ICAAC Meeting*, Toronto, Canada (Abstract No. 2177)
33 Thomas, W. *et al.* (2000) Discovery and *in vitro* enzyme activity of BB3497 – A new class of peptide deformylase (PDF) inhibitor. *40th Annual ICAAC Meeting*, Toronto, Canada (Abstract No. 2176)
34 Clements, J.M. *et al.* (2001) Antibiotic activity and characterization of BB3497, a novel peptide deformylase inhibitor. *Antimicrob. Agents Chemother.* 45, 563–570
35 Jayasekera, M.M.K. *et al.* (2000) Novel nonpeptidic inhibitors of peptide deformylase. *Arch. Biochem. Biophys.* 381, 313–316
36 Hu, Y.J. *et al.* (1998) H-phosphonate derivatives as novel peptide deformylase inhibitors. *Bioorg. Med. Chem. Lett.* 8, 2479–2482
37 Leung, D. *et al.* (1999) Protease inhibitors: current status and future prospects. *J. Med. Chem.* 43, 305–341
38 Petrillo, E.W., Jr and Ondetti, M.A. (1982) Angiotensin-converting enzyme inhibitors: medicinal chemistry and biological actions. *Med. Res. Rev.* 2, 1–41
39 Rasmussen, H.S. and McCann, P.P. (1997) Matrix metalloproteinase inhibition as a novel anticancer strategy: a review with special focus on Batimastat and Marimastat. *Pharmacol. Ther.* 75, 69–75
40 Becker, A. *et al.* (1998) Structure of peptide deformylase and identification of the substrate binding site. *J. Biol. Chem.* 273, 11413–11416

41 Meinnel, T. *et al.* (1996) A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase. *J. Mol. Biol.* 262, 375–386
42 Chan, M.K. *et al.* (1997) Crystal structure of the *Escherichia coli* peptide deformylase. *Biochemistry* 36, 13904–13909
43 Hao, B. *et al.* (1999) Structural basis for the design of antibiotics targeting peptide deformylase. *Biochemistry* 38, 4712–4719
44 Dardel, F. *et al.* (1998) Solution structure of nickel-peptide deformylase. *J. Mol. Biol.* 280, 501–513
45 Hackbarth, C.J. *et al.* (2000) Microbiological and enzymatic evaluation of deformylase inhibitors. *40th Annual ICAAC Meeting,* Toronto, Canada (Abstract No. 2173)
46 Gordon, J.J. *et al.* (1962) Actinonin: an antibiotic substance produced by an Actinomycete. *Nature* 195, 701–702
47 Apfel, C.M. *et al.* (2001) Peptide deformylase as an antibacterial drug target: target validation and resistance development. *Antimicrob. Agents Chemother.* 45, 1058–1064
48 Broughton, B.J. *et al.* (1975) Studies concerning the antibiotic actinonin. Part VIII. Structure–activity relationships in the actinonin series. *J. Chem. Soc., Perkin Trans.* 9, 857–860
49 Green, B.G. *et al.* (2000) Inhibition of bacterial peptide deformylase by biaryl acid analogs. *Arch. Biochem. Biophys.* 375, 355–358
50 Durand, D.J. *et al.* (1999) Peptide aldehyde inhibitors of bacterial peptide deformylases. *Arch. Biochem. Biophys.* 367, 297–302

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