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The evolution of peptide deformylase as a target: Contribution of biochemistry, genetics and genomics

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ABSTRACT

Although peptide deformylase (PDF, EC 3.5.1.27) was first described in 1968, the instability of enzyme preparations prevented it from being seriously considered as a target until this problem was finally solved in 1998. PDFs essentiality was first demonstrated in *Escherichia coli* in 1994. Genomic analyses have shown this enzyme to be present in all eubacteria. PDF homologs have also been found in eukaryotes including *Homo sapiens*. The function and relevance of the human chromosomal homolog to the safety of PDF inhibitors as therapeutic agents is not clear at this stage. Although there is considerable sequence variation between the different bacterial PDFs, there are three strongly conserved motifs that together constitute a critical metal binding site. The observation that PDF is a metalloenzyme has led to the design of inhibitors containing metal chelating pharmacophores. The most potent of these synthetic inhibitors are active against a range of clinically relevant respiratory tract pathogens in vitro and in vivo, including those resistant to current antibiotics. Mutants resistant to PDF inhibitors have been obtained in the laboratory; these resulted from mutations in the genes for transformylase (EC 2.1.2.9) or PDF. The mechanism involved and its frequency were pathogen-dependent. The two most advanced PDF inhibitor leads, which are both reverse hydroxamates, have progressed to phase 1 clinical trials and were well tolerated.

1. Discovery of PDF

In 1966 Capecchi [1] proposed that *N*-formyl methionine was a universal initiator in bacterial protein synthesis. However, in the vast majority of cases the formyl group is cleaved off and in many cases the methionine is removed as well. Adams [2] was the first to demonstrate the presence of PDF (EC 3.5.1.27) activity in a bacterial extract; he noted the instability of the enzyme and its inhibition by mercaptoethanol. Although we now know that methionine plays a key role in the initiation of protein synthesis in all cells, the formylation and subsequent deformylation of methionine is peculiar to prokaryotes. It plays no role in the cytosolic protein synthesis of eukaryotes. The presence and role of these enzymes in cellular organelles, such as mitochondria, will be discussed later. All this biochemical information already suggested by the late 1960s the suitability of PDF as a potential target for antibacterial agents. However, proof of essentiality was lacking, and the instability of PDF was a serious obstacle.

2. PDF defined as an antibacterial target

Further progress on PDF had to wait more than 20 years and depended on advances in bacterial genetics and molecular biology. In 1993 the gene coding for PDF in *Escherichia coli* was

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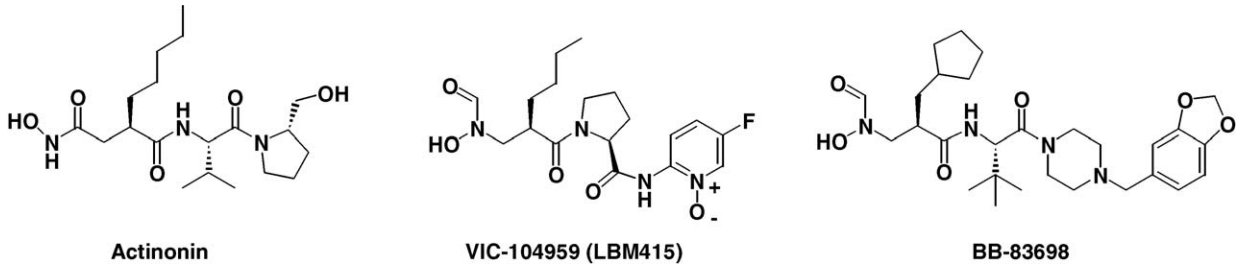

Fig. 1 – Structure of PDF inhibitors. Actinonin is a natural product isolated in 1962 with an unknown mechanism of action that was subsequently shown to be a PDF inhibitor in 2000. LBM415 and BB-83698 are synthetic PDF inhibitors that have both progressed to phase 1 clinical trials in human volunteers.

shown to belong to a single operon with the related formyl transferase (FMT; EC 2.1.2.9) gene [3]. When PDF was over-expressed and purified it was found to contain a tightly bound zinc atom (in agreement with the predicted presence of a zinc binding motif in the protein). However, the enzyme obtained had a very low specific activity. In 1994 further characterization of the PDF- and FMT-encoding genes showed that PDF was essential whereas knocking out FMT only resulted in impaired growth [4]. PDF could for the first time legitimately described as a potential target for antibacterials. Interestingly, although it is essential in an otherwise normal background, the PDF gene could be knocked out, provided that the FMT gene was knocked out as well. Such double mutants, like the FMT single mutants, were seriously growth impaired. In 1995 it was shown that PDF activity was inhibited by phenanthroline [5], a further indication of the importance of metal ions to enzyme activity. These results were reinforced by Pei's laboratory [6], which reported that several divalent metal ion chelators were potent inhibitors of PDF.

3. Solving the problem of PDF instability

The enduring problem of PDF instability was finally solved in 1998 when it was shown that the natural metal is actually ferrous ion and not zinc [7]. The ferrous ion-containing enzyme is highly active but unstable. Excluding oxygen from purification solutions stabilized enzyme activity by preventing the oxidation of the active ferrous ion to the inactive ferric form. Over-expression and purification using standard conditions results in formation of a poorly active enzyme containing zinc, which is assumed to be an artifact. It is not clear if the zinc enzyme itself possesses poor activity or whether the small amount of activity observed is due to traces of other active divalent cations. Fortunately, it was discovered that addition of other divalent cations, such as cobalt or nickel, during purification made it possible to isolate highly active, stable preparations of PDF [8,9]. These practical observations paved the way for a number of groups to initiate the synthesis of PDF inhibitors.

4. Application of mechanism-based design to PDF inhibition

The fact that PDF is a metalloenzyme suggested use of an approach to inhibitor design pioneered by researchers at Squibb. Their work on the human angiotensin converting enzyme (ACE; EC 3.4.15.1) led to the synthesis of the selective inhibitor Captopril®, which is used for the treatment of hypertension [10]. ACE is a metalloenzyme; Captopril® selectively chelates the metal ion present at the active site, inhibiting enzyme activity. The chelating function in Captopril® is a sulfhydryl group. This mechanism-based drug design approach was subsequently applied to PDF. A moiety resembling the enzyme substrate is coupled with an appropriate chelator. Researchers at Vicuron Pharmaceuticals recognized that a molecule having such characteristics had already been described: actinonin [11]. This natural product (see Fig. 1) had been discovered in 1962 [12] by empirical screening and, although it had antibacterial activity, its mechanism of action was unknown. In 1999 in vitro tests with purified PDF confirmed that actinonin was indeed a potent inhibitor of the enzyme [11]. The chelating group present in actinonin is a hydroxamate. A range of different chelators has been tried in the quest to synthesize PDF inhibitors including hydroxamates, reverse hydroxamates, and carboxylates, as well as sulfhydryls. At this stage the most potent and selective leads reported contain reverse hydroxamates. Many other important enzymes in human cells are also dependent on metal ions for their function; thus there is a risk that PDF inhibitors of this type would not provide sufficient selectivity and therefore be toxic. The mammalian matrix metalloproteases are a well-known group of enzymes that are being pursued as potential anticancer targets [13]. The availability of such enzymes, in addition to ACE, provided a suitable panel to check the selectivity of PDF inhibitors at the in vitro level. In addition, the pursuit of matrix metalloprotease inhibitors using the same mechanism-based drug design approach provided relevant insight for the design of PDF inhibitors. The first crystal structure for PDF from *E. coli* was published in 1997 [14], and further reports with and without inhibitors quickly followed [8,15,16]. The availability of this structural information has undoubtedly provided additional input on which to base inhibitor design. For a more thorough discussion of PDF inhibitors see Jain et al. [17].

5. Activity against intact bacteria

Identification of a potent PDF inhibitor is no guarantee that it will be active against intact bacterial cells. The ability of an inhibitor to access the target (by penetrating the permeability