Patatin-like proteins: a new family of lipolytic enzymes present in bacteria?

Patatins are a group of plant storage glycoproteins that show lipid acyl hydrolase activity (Andrews et al., 1988). The patatin-associated lipolytic activity may be a means of defence against plant parasites (Strickland et al., 1995) and has been shown to function in plant signal transduction (Holk et al., 2002). Hirschberg et al. (2001) found that potato patatin B2 and human cytosolic phospholipase A2 (cPLA2) share conserved domains of protein homology and therefore predicted that patatin B2, like cPLA2, contains an active-site dyad instead of the more common Ser-His-Asp (or Glu) triad of lipolytic enzymes (Schrag & Cygler, 1997). This was confirmed by the crystal structure of the potato patatin isozyme Pat17 which together with mutagenesis studies revealed that the active site consists of a Ser-Asp dyad (Rydell et al., 2003). Recently, Sato et al. (2003) and Phillips et al. (2003) have shown that ExoU, a type III secreted cytotoxin and virulence factor of Pseudomonas aeruginosa with lipase as well as phospholipase A activity, contains defined regions of protein homology to potato patatin B2 and cPLA2 with a putative catalytic Ser-Asp dyad. In animal models and human infections, the toxicity of ExoU has been linked to the development of lung injury, sepsis and bacterial dissemination (Allewelt et al., 2000; Finck-Barbançon et al., 1997; Hauser et al., 1998; Kurahashi et al., 1999). Thus, ExoU is both an important virulence factor of P. aeruginosa and the first patatin-like bacterial hydrolase characterized. However, it has yet to be determined whether patatin-like proteins (PLPs) are found in bacteria other than P. aeruginosa.

To find out whether PLPs exist in bacteria other than P. aeruginosa, we searched (A) all bacterial genomes available for patatin B2 and P. aeruginosa ExoU homologues by means of the BLAST algorithm (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) (Altschul et al., 1997) and (B) we applied the key word ‘patatin’ to screen the 123 accessible completed bacterial genomes using the text search tool of the PEDANT server (http://pedant.gsf.de). The PEDANT server provides extensive data from computational analysis of genome sequences by algorithms such as PROSITE, PFAM, BLAST and others. For search (A), the BLAST alignment, we only obtained a minimal number of hits for PLPs in bacteria (<20 for patatin B2 or ExoU, BLAST expect value ≤1). On the other hand, we found a high number of hits (>200) when we used the PEDANT server text search. In 134 proteins (PFAM domain expect value ≤0.5), text search hits for ‘patatin’ originated from the detection of the patatin domain by the PFAM algorithm (Bateman et al., 2002), indicating that only short amino acid sequences of homology were conserved within related proteins. This might also explain why the application of the BLAST algorithm did not lead to adequate results. Genes encoding PLPs were detected in 55 of the 123 completed bacterial genomes including a wide range of Gram-positive and Gram-negative bacteria, such as Bacillus sp., Brucella sp., Rickettsia sp., Staphylococcus aureus, Yersinia pestis and many others.

Moreover, we observed that particularly some animal pathogen and plant pathogen/symbiont genomes contain a high number of PLP genes, e.g. Bradyrhizobium japonicum (n = 8), Leptospira interrogans (n = 6), Mesorhizobium loti (n = 6), Mycobacterium tuberculosis (n = 8) and Ralstonia solanacearum (n = 5). We were interested in whether pathogens/symbionts possess more PLP genes than non-pathogens. Thus, we compared the number of PLP genes present per 1000 open reading frames (ORFs) in the two groups and found that the genomes of the pathogens/symbionts showed a significantly higher number of PLP genes than the genomes of non-pathogens, more precisely 0.80 ± 0.50 and 0.53 ± 0.24 (P ≤ 0.01, Student’s t-test), respectively. The number of PLP genes also varied between different species of a genus. For example, the genome of...
Fig. 1. Alignment of selected bacterial PLPs with conserved patatin domains. Sixty proteins with patatin-like domains (PFAM domain expect value \(< 5 \times 10^{-19}\)) were aligned by the CLUSTAL W method (Thompson et al., 1994) using the program MEGALIGN (DNASTAR). Conserved protein domains of a selection of PLPs (PFAM domain expect values from 6 to 38) representing different bacterial genera and including proteins with different N- or C-terminal extensions are listed in the order of increasing PFAM expect values. Residues which were conserved in more than 80% of the 60 aligned proteins are marked bold. An arrow designates the members of the catalytic dyad in patatin B2 (Rydel et al., 2003). Δ, The number of amino acid residues present before and after the conserved blocks. Conserved regions of patatin B2 (modified from Hirschberg et al., 2001) and of P. aeruginosa ExoU are shown for comparison.
Mycobacterium leprae included only one PLP gene, whereas the genome of M. tuberculosis contained eight corresponding genes. This finding is in agreement with a previous report which stated that M. leprae originated by a process of reductive evolution resulting in a minimal gene set sufficient for survival in its highly specialized niche (Cole et al., 2001). In contrast, 52% of the proteome of M. tuberculosis was derived from gene duplication (Tekaria et al., 1999), leading to a higher flexibility in utilizing nutrients. Furthermore, the genome of pathogenic Escherichia coli O157 : H7 encoded a higher number of PLP genes (n = 4) than the genome of non-pathogenic strain E. coli K-12 (n = 2), which again implies a correlation between the number of PLP genes and virulence. Hence, we speculate that a high number of PLP genes might confer an advantage to the bacterium in (i) interaction with the host, (ii) adaptation to various environments and (iii) competition with other micro-organisms.

To determine whether the bacterial PLPs share all characteristic domains with their eukaryotic counterparts, 60 bacterial PLPs (PFAM domain expect value ≤5.5 × 10⁻¹⁹) were aligned by the CLUSTAL W method (Thompson et al., 1994). Bacterial PLPs were found to possess four conserved domains (block I–IV) similar to those in potato patatin B2 (Fig. 1). Block I consists of a glycine-rich region with a conserved arginine or lysine residue which probably serves as an oxyanion hole. Block II is located in proximity to block I (about 10–20 aa distance) and comprises the hydrolase motif G-X-S-X-G with the putative active-site serine (Schrag & Cygler, 1997). Block III contains a conserved serine, which may be an important structural element as a potential phosphorylation site or due to its capacity to form hydrogen bonds. The adjacent highly conserved proline residues (in blocks III and IV) may be important for the proper conformation of the protein. In addition to many structural features shared both by eukaryotic and bacterial PLPs, we nevertheless observed that in block III bacterial and eukaryotic PLPs show different conserved motifs. While bacterial PLPs possessed the conserved sequence A-S-X-X-P, eukaryotic patatin homologues contained the conserved amino acids A-P, as seen by CLUSTAL W alignment of 20 eukaryotic PLPs (Fig. 1, and data not shown). Block IV includes the putative active-site aspartate which is the second member of the catalytic dyad in patatin Pat17 (Rydel et al., 2003). Successive to the domain with the active-site aspartate, Hirschberg et al. (2001) have defined an additional region of protein homology for potato patatin B2 and human cPLA₂ containing a conserved serine. We found that this domain was present in at least 15 other eukaryotic patatin homologues (data not shown) but was not conserved in the respective prokaryotic proteins (Fig. 1).

We therefore conclude that eukaryotic and bacterial PLPs share many but not all structural features, suggesting that they originated from a common ancestor and were modified to support/expand the specific lifestyle of an organism.

Arpigny & Jaeger (1999) classified bacterial esterases and lipases into eight groups based on their amino acid sequence. Bacterial PLPs do not show any homology to known groups of bacterial lipases as evaluated by BLAST (Altschul et al., 1997) or PFAM domain search (Bateman et al., 2002) (data not shown), indicating that the amino acid sequences of PLPs and established groups of lipolytic enzymes are not closely related. Second, except for the G-X-S-X-G lipase motif which is common even in distinct lipase families, the conserved domains presented here for bacterial PLPs do not exhibit the same features as found for other bacterial lipases (Fig. 1) (Arpigny & Jaeger, 1999). Finally, the lipases classified by Arpigny & Jaeger (1999) either possess a catalytic Ser-Asp-His triad or a Ser-His dyad which makes the putative Ser-Asp catalytic dyad of patatin homologues unique among bacterial lipases (Rydel et al., 2003). Since the prokaryotic PLPs appear to be more related to their eukaryotic counterparts than to any other group of bacterial lipases, we propose that they comprise a new group of bacterial lipolytic enzymes.

Finally, while comparing the size of bacterial PLPs, we noticed that several of them possess a N- or C-terminal extension (<300 aa) preceding or successive to the conserved blocks, respectively (Fig. 1). An additional domain search revealed that many PLPs are two- or multi-domain proteins. Some bacterial proteins with N-terminal extensions showed a predicted cyclic nucleotide-binding domain (e.g. Caulobacter crescentus gi13423668, L. interrogans gi24214875, M. tuberculosis gi13883156) and therefore might be regulated by cAMP or cGMP (McCue et al., 2000). Several of the proteins with C-terminal extensions possessed a putative bacterial-surface-antigen domain (e.g. Pseudomonas putida gi26991199, Vibrio cholerae gi9650535, Vibrio vulnificus gi27361160), which is also present in outer-membrane proteins such as D15 from Haemophilus influenzae (Loosmore et al., 1997). Furthermore, P. aeruginosa ExoU (gi2429143) contains a C-terminal extension, which has been described to be essential for its cytotoxic activity (Finck-Barbançon & Frank, 2001; Hauser et al., 1998).

We have herein presented evidence that genes of PLPs are found in many bacterial genomes. Therefore, it is conceivable that many bacteria possess as-yet-unidentified lipolytic enzymes. Since these proteins show catalytic domains and conserved regions distinct from all groups of lipolytic enzymes known so far in bacteria, we believe that they comprise a new group of hydrolytic enzymes. With the exception of P. aeruginosa cytotoxin ExoU, none of the bacterial PLPs have been characterized at present. Consequently, the identification of the patatin domain may be a valuable clue for characterizing these putative hydrolases and could help discover new virulence factors.

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Sangeeta Banerji and Antje Flieger
Robert Koch-Institut, Nordufer 20, D-13353 Berlin, Germany

Correspondence: Antje Flieger
(fliegera@rki.de)


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