Cloning and characterization of the NapA acid phosphatase/phosphotransferase of *Morganella morganii*: identification of a new family of bacterial acid-phosphatase-encoding genes

Maria Cristina Thaller, Giovanna Lombardi, Francesca Berlutti, Serena Schippa, and Gian Maria Rossolini

Author for correspondence: G. M. Rossolini. Tel: +39 577 280903. Fax: +39 577 42011.

The gene encoding a minor phosphate-irrepressible acid phosphatase (named NapA) of *Morganella morganii* was cloned and sequenced, and its product characterized. NapA is a secreted acid phosphatase composed of four 27 kDa polypeptide subunits. The enzyme is active on several organic phosphate monoesters but not on diesters, and is also endowed with transphosphorylating activity from organic phosphoric acid esters to nucleosides and other compounds with free hydroxyl groups. Its activity is inhibited by EDTA, inorganic phosphate, nucleosides and Ca$^{2+}$, but not by fluoride or tartrate, and is enhanced by Mg$^{2+}$, Co$^{2+}$ and Zn$^{2+}$. At the sequence level, the NapA enzyme did not show similarities to any other sequenced bacterial phosphatases. However, a search for homologous genes in sequence databases allowed identification of two open reading frames located within sequenced regions of the *Escherichia coli* and *Proteus mirabilis* genomes respectively, encoding proteins of unknown function which are highly homologous to the *Morganella* enzyme. Moreover, the properties of the NapA enzyme are very similar to those reported for the periplasmic nonspecific acid phosphatase II of *Salmonella typhimurium* (for which no sequence data are available). These data point to the existence of a new family of bacterial acid phosphatases, which we propose designating class B bacterial acid phosphatases.

**Keywords:** *Morganella morganii*, NapA, acid phosphatase/phosphotransferase

**INTRODUCTION**

Enzymic cleavage of phosphoester bonds is a fundamental step in many biochemical pathways and several phosphatases, different in structure and function are typically present in the prokaryotic cell (Beacham, 1979; Dvorak et al., 1967; Uerkvitz & Beck, 1981). However, even in *Escherichia coli*, which is the best studied model of such cells, the exact number of phosphatases as well as the role(s) of some of them remain to be defined. The picture is further complicated by the fact that different patterns of phosphatase activities are found in different bacterial species, even those belonging to closely related taxa, as observed for members of the family *Enterobacteriaceae* (Cocks & Wilson, 1972; Edwards et al., 1993; Groisman et al., 1992; Pompei et al., 1990, 1993).

Characterization of the different phosphatases present in enteric bacteria is, therefore, interesting not only in relation to microbial physiology and enzymology but also enterobacterial taxonomy and phylogenesis.

In a previous investigation on the phosphatases produced by *Morganella morganii*, which is one of the few enterobacterial species able to produce a high-level phosphate-irrepressible acid phosphatase activity (HPAP phenotype) (Pompei et al., 1990, 1993), we found that *Morganella* produces two different phosphate-irrepressible acid phos-
phosphatases active on p-nitrophenyl phosphate (pNPP), and that the major one, named PhoC, is apparently associated with the HPAP phenotype (Thaller et al., 1994). PhoC is a nonspecific acid phosphatase homologous to other bacterial acid phosphatases, including the PhoN enzymes of Salmonella typhimurium and Providencia stuartii, and the PhoC enzyme of Zymomonas mobilis, and we proposed designating this family of enzymes class A bacterial acid phosphatases (Thaller et al., 1994).

In the present work we have studied the minor phosphate-irrepressible acid phosphatase produced by M. morganii, named NapA, and found that it belongs to another family of nonspecific acid phosphatases which could be widespread among the Enterobacteriaceae and which we propose designating class B bacterial acid phosphatases.

METHODS

Bacterial strains and genetic vectors. The M. morganii RS12 strain used for library construction has been previously described (Thaller et al., 1994). M. morganii ATCC 25830T was also used as a reference strain. E. coli DH5α (Sambrook et al., 1989) was used as the host for genetic vectors and recombinant plasmids. The Bluescript SK plasmid (Stratagene) was the vector used for the construction of the M. morganii genomic library and for subcloning procedures.

Recombinant DNA methodology. Basic recombinant DNA techniques were essentially as described by Sambrook et al. (1989). The procedure for construction of the Morganella genomic library has been previously described (Thaller et al., 1994). All sequences were determined on denatured double-stranded DNA templates by the dideoxy chain-termination method (Sanger et al., 1977). The nucleotide sequence was determined for both strands. Analysis and comparison of nucleotide and amino acid sequences were performed using the UWGCG software (release 7.3) (Devereux et al., 1984).

Phosphatase assays. The phosphatase activity of whole-cell or periplasmic protein preparations toward pNPP was assayed by measuring the released p-nitrophenol (pNP) at 414.5 nm and pH 12. The concentration of pNP in the assay was 5 mM. All assays were performed in a volume of 1 ml and were initiated by the addition of the substrate. Incubation was at 37 °C for 20 min. One unit of enzyme activity was defined as the amount of enzyme able to release 1 μmol pNP min⁻¹ under the assay conditions.

For preparation of whole-cell proteins, bacteria were washed twice in normal saline, suspended in normal saline at an OD₅₀₀ of 10, and disrupted by sonication. Cell debris was then removed by centrifugation (10,000 g for 10 min at 4 °C). Extraction of periplasmic proteins from E. coli was performed by chloroform treatment (Ferro-Luzzi Ames et al., 1984).

Determination of the pH optimum of the purified NapA enzyme was performed using pNPP as substrate in 100 mM buffers, including sodium acetate buffer (pH 4-6), Tris/HCl buffer (pH 7-9), and glycine/NaOH buffer (pH 10). Measurement of enzyme activity in different buffer systems at overlapping pH values showed no significant buffer-related variation. Determination of the activity of the purified NapA protein using different substrates was performed as previously described (Kier et al., 1977; Weppelman et al., 1977) in 100 mM sodium acetate buffer, pH 6. Inhibition assays were performed in the same buffer using pNPP as the substrate. The enzyme was pre-incubated at 37 °C for 30 min with each substance before starting the assay. Phosphotransferase activity was assayed in 100 mM sodium acetate buffer, pH 6, as previously described (Uerkvitz, 1988), using pNPP as the phosphate donor.

Protein determination. Protein concentration in solution was determined using a commercial kit (Bio-Rad Protein assay). BSA was used as the standard.

Protein electrophoretic techniques. SDS-PAGE was performed as described by Laemmli (1970). For detection of NapA enzyme activity after electrophoresis, the gel was incubated for 4 h at 37 °C in several changes of renaturation buffer to obtain renaturation of the enzyme. Renaturation buffer was 100 mM Tris/HCl, pH 7, containing 5 mM MgSO₄ and 1% (v/v) Triton X-100. After the renaturation treatment, the gel was equilibrated for 1 h in 100 mM sodium acetate buffer, pH 6, containing 5 mM MgSO₄, and then developed for phosphatase activity. For development, the gel was incubated at 37 °C for 30 min in the same buffer as used for equilibration with 5 mM pNPP added, washed in deionized water, and then incubated at 42 °C in a freshly prepared solution made by a 6:1 (v/v) mixture of acidified ammonium molybdate (4.2 g ammonium molybdate l⁻¹ and 28.6 ml sulphuric acid l⁻¹) and 10% (w/v) ascorbic acid, to detect the presence of inorganic phosphate (Ames, 1966). Phosphatase activities were indicated by the presence of blue-stained bands.

Purification of the NapA enzyme. When E. coli strains producing the Morganella NapA enzyme were grown in liquid medium at 37 °C, a consistent amount of the protein was released into the medium. The NapA protein was purified from the supernatant of a 24-h-old stationary-phase culture of clone PM9 (see below) grown at 37 °C in Brain Heart Infusion broth supplemented with carbenicillin (0.2 mg ml⁻¹). The culture supernatant was dialysed against 10 mM Tris/HCl, pH 7-4, and loaded on a DEAE-Sepharose CL-6B (Pharmacia) column (15 x 11.5 cm) equilibrated with the same buffer. The enzyme did not bind to the resin and eluted as a single peak. Fractions containing the eluted enzyme were collected, pooled, and dialysed against 10 mM HEPES, pH 7-4. The enzyme was then loaded on a CM-Sephadex C-50 (Pharmacia) column (1.5 x 11.5 cm) equilibrated with 10 mM HEPES, pH 7-4. Again, the enzyme did not bind to the resin and eluted as a single peak. Fractions containing the eluted enzyme were pooled, dialysed against 100 mM sodium acetate buffer, pH 6, and concentrated by ultrafiltration in a Centricon 10 concentrator (Amicon). At this point the enzyme was > 95% pure, as assayed by SDS-PAGE, and only two additional bands were evident. Removal of these contaminants was obtained by gel-filtration. For this purpose, SDS was added to a final concentration of 2% (w/v) and the enzyme was immediately loaded (maximum loading volume 0.3 ml) onto a Sephacryl S-200 (Pharmacia) column (1 x 40 cm) equilibrated with 100 mM sodium acetate buffer, pH 6. The enzyme eluted as a single peak and the fractions containing it were pooled, dialysed against 100 mM sodium acetate buffer, pH 6, and concentrated by ultrafiltration as described above.

For determination of the molecular mass of the NapA protein by means of gel-filtration, the purified protein was applied to a Sephacryl S-200 column (1 x 40 cm) equilibrated and eluted with 100 mM sodium acetate buffer, pH 6. The Sephacryl S-200 column was calibrated with protein standards (Pharmacia) dissolved in elution buffer. The peak fraction of each protein was determined by A₂₈₀. A linear plot of the partition coefficients (Reiland, 1971) versus the logs of the molecular masses of the protein standards was used to estimate the molecular mass of the phosphatase.
**RESULTS**

Cloning of the *M. morganii* genetic determinant encoding the minor phosphate-irrepressible acid phosphatase (NapA)

The *M. morganii* gene coding for the minor phosphate-irrepressible acid phosphatase (named NapA) was isolated from a genomic library of *M. morganii* RS12, constructed in the plasmid vector Bluescript SK and transformed in *E. coli* DH5α by the same shotgun cloning strategy previously used for isolation of the *Morganella phoC* gene (Thaller et al., 1994).

Using this strategy, two clones (PM2 and PM9) were identified, which showed acid phosphatase activity much higher than *E. coli* DH5α(pBluescript) (data not shown) and produced an acid phosphatase composed of a 27 kDa polypeptide which was not detectable in *E. coli* (Fig. 1a) and apparently corresponded to the minor phosphate-irrepressible acid phosphatase detectable in *Morganella* by a zymogram technique (Fig. 1b). The acid phosphatase produced by the two clones could be extracted by a chloroform treatment suitable for extraction of the periplasmic proteins from *E. coli* (Ferro-Luzzi Ames et al., 1984) (Fig. 1c) and the ratio of phosphatase activity between a periplasm and a whole cell protein preparation was consistent with a location of this protein in the periplasmic space (data not shown).

Restriction mapping and cross-hybridization experiments performed using the insert carried by clone PM9 as a probe showed that the two clones carried overlapping DNA fragments (Fig. 2), which a Southern blot analysis confirmed to be derived from the *M. morganii* RS12 genome (data not shown).

The above results suggested that the *Morganella* gene encoding the minor phosphate-irrepressible acid phosphatase had been cloned. Definitive confirmation that the enzyme produced by PM2 and PM9 was actually encoded by the cloned DNA was obtained by comparison of the NH₂-terminal sequencing data of the enzyme with the nucleotide sequence of the cloned DNA (see below).

**Characterization of the NapA enzyme**

Purification of the *Morganella* NapA enzyme from the culture supernatant of *E. coli* PM9 was achieved by means of two ion-exchange chromatography steps followed by a gel-filtration step (see Methods for details of the purification procedure). The two ion-exchange steps were able to remove most protein contaminants from the initial preparation (the NapA enzyme did not bind to either of the two matrices under the conditions adopted), while the final gel-filtration step allowed purification to homogeneity of the NapA protein, as judged by SDS-PAGE (Fig. 3a). The specific activity of the purified protein, assayed against pNPP at pH 6, was 46.2 U mg⁻¹.

The NH₂-terminal sequence of the NapA protein was determined as NH₂-KVYMPEKVSDBGVTVAQLAEQ.

The molecular mass of the protein, estimated by gel-filtration chromatography, was approximately 105 kDa. This finding was confirmed by ultrafiltration experiments, which showed that the protein was completely retained by filters which excluded globular proteins of sizes larger than 100 kDa.

The NapA enzyme was completely denatured to the monomeric form following heating in the presence of 2% SDS (Fig. 3b). However, avoiding the heat treatment before loading the SDS-PAGE gel resulted in only partial denaturation of the protein, part of which migrated as an approximately 100 kDa band (Fig. 3b). It was possible to vary the ratio between the 100 kDa and the 27 kDa forms by varying the duration of the exposure to SDS and/or the degree of heat treatment of the protein sample before loading the gel (data not shown). If heating was avoided and, at the same time, either the SDS concentration was lowered to 0.01%, or 1% Triton X-100 was added, the protein migrated almost exclusively as a 100 kDa band (Fig. 3b). Results of partially denaturing SDS-PAGE, which gave a molecular mass of approximately 100 kDa
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NapA clone/subclone production
SB RISTI/RI SC B I A SB
- t-pPM2 + SB B - m RI B, Sm B - napnodi, 1 kb pPM9 pPM9/03R pPM9/12R - pPM9/13Sm -

Fig. 2. Restriction endonuclease map of the DNA inserts of the recombinant plasmids carried by clones PM2 and PM9 (respectively named pPM2 and pPM9) and subcloning strategy. SB, Sau3AI/BamHI junction; RI, EcoRI; Sm, SmaI; Sc, SacI; B, BamHI; A, Apal. Thick lines represent Morganella DNA while thin zigzag lines represent vector sequences. The location of the putative NapA ORF identified on the basis of sequencing data is shown below the map. Production of the NapA protein was assayed both by measuring pNPP-hydrolysing activity at pH 6, and by SDS-PAGE analysis of periplasmic proteins of different subclones.

Fig. 3. SDS-PAGE analysis of the purified NapA protein. (a) Purified NapA protein (approximately 5 µg); the gel was stained with a silver staining technique (Heukeshoven & Dernick, 1985) allowing a detection sensitivity of at least 5 ng of protein per band. (b) Coomassie-blue-stained preparation of the purified NapA protein subjected to SDS-PAGE after different treatments before loading: the protein was mixed with Laemmli’s sample buffer in the presence of 2% SDS and boiled for 5 min (lane 1), mixed with Laemmli’s sample buffer in the presence of 2% SDS and 1% Triton X-100 and incubated at 25 °C for 5 min (lane 2), mixed with Laemmli’s sample buffer in the presence of 0.01% SDS and incubated at 25 °C for 5 min (lane 3), or mixed with Laemmli’s sample buffer in the presence of 2% SDS and incubated at 25 °C for 5 min (lane 4). Protein size markers are given in kDa on the left.

Table 1. pNPP-hydrolysing activity of the NapA phosphatase at different pH values

<table>
<thead>
<tr>
<th>pH</th>
<th>pNP production (nmol min⁻¹ ml⁻¹)</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7.86 ± 0.13</td>
<td>0.60</td>
</tr>
<tr>
<td>4.5</td>
<td>10.22 ± 0.15</td>
<td>0.78</td>
</tr>
<tr>
<td>5</td>
<td>10.74 ± 0.15</td>
<td>0.82</td>
</tr>
<tr>
<td>5.5</td>
<td>11.53 ± 0.17</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>13.10 ± 0.18</td>
<td>1.00</td>
</tr>
<tr>
<td>6.5</td>
<td>12.45 ± 0.19</td>
<td>0.95</td>
</tr>
<tr>
<td>7</td>
<td>11.40 ± 0.17</td>
<td>0.87</td>
</tr>
<tr>
<td>8</td>
<td>5.11 ± 0.10</td>
<td>0.39</td>
</tr>
<tr>
<td>9</td>
<td>1.70 ± 0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>0.39 ± 0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Mean value of three experiments ± SE.

Table 1. pNPP-hydrolysing activity of the NapA phosphatase at different pH values

All assays were performed using 0.3 µg of the purified enzyme in a volume of 1 ml.

more than 50% of maximal activity in the pH range 4–7 (Table 1). The enzyme showed a broad substrate specificity which did not include diesters (Table 2). The activity of the enzyme was inhibited by EDTA, inorganic phosphate and Ca²⁺, stimulated by Mg²⁺, Co²⁺ and Zn²⁺, and apparently unaffected by fluoride and tartrate (Table 3). NapA also showed transphosphorylating activity from organic phosphoric acid esters to nucleosides and other compounds with free hydroxyl groups (Table 4). Results of these experiments also showed that the activity of NapA was inhibited by nucleosides and stimulated by high concentrations of ethanol and glycerol.

The functional properties of the NapA enzyme, as well as the sizes of the native protein and of its polypeptide component, are very similar to those of the S. typhimurium NapII enzyme (Uerkvitz, 1988; Uerkvitz & Beck, 1981).
Table 2. Relative activities of the NapA phosphatase toward various substrates

All assays were performed using 0.3 μg of the purified enzyme in a volume of 1 ml.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>P_i production (nmol min⁻¹ ml⁻¹)*</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-AMP</td>
<td>10.37±0.21</td>
<td>1.00</td>
</tr>
<tr>
<td>5'-UMP</td>
<td>2.90±0.08</td>
<td>0.28</td>
</tr>
<tr>
<td>3'-AMP</td>
<td>12.34±0.23</td>
<td>1.19</td>
</tr>
<tr>
<td>3'-UMP</td>
<td>6.95±0.14</td>
<td>0.67</td>
</tr>
<tr>
<td>pNPP</td>
<td>12.86±0.25</td>
<td>1.24</td>
</tr>
<tr>
<td>PDP</td>
<td>11.82±0.20</td>
<td>1.14</td>
</tr>
<tr>
<td>G2P</td>
<td>5.08±0.11</td>
<td>0.49</td>
</tr>
<tr>
<td>Glucose 6-P</td>
<td>2.07±0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Ribose 5-P</td>
<td>6.62±0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>2':3'-Cyclic AMP</td>
<td>&lt;0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2':3'-Cyclic UMP</td>
<td>&lt;0.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Mean value of three experiments ± SE.

Table 3. Effect of various substances on the pNPP-hydrolysing activity of the NapA enzyme

All assays were performed using 0.3 μg of the purified enzyme in a volume of 1 ml.

<table>
<thead>
<tr>
<th>Substance (mM)</th>
<th>pNP production (nmol min⁻¹ ml⁻¹)*</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12.50±0.19</td>
<td>1.00</td>
</tr>
<tr>
<td>EDTA (1)</td>
<td>1.25±0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>EDTA (20)</td>
<td>&lt;0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ATP (20)</td>
<td>12.75±0.18</td>
<td>1.02</td>
</tr>
<tr>
<td>P_i (50)</td>
<td>10.25±0.16</td>
<td>0.82</td>
</tr>
<tr>
<td>P_i (100)</td>
<td>7.75±0.14</td>
<td>0.62</td>
</tr>
<tr>
<td>Tartrate (1)</td>
<td>12.88±0.18</td>
<td>1.03</td>
</tr>
<tr>
<td>Tartrate (10)</td>
<td>12.38±0.19</td>
<td>0.99</td>
</tr>
<tr>
<td>F⁻ (1)</td>
<td>11.88±0.17</td>
<td>0.95</td>
</tr>
<tr>
<td>F⁻ (10)</td>
<td>11.50±0.16</td>
<td>0.92</td>
</tr>
<tr>
<td>Ca²⁺ (1)</td>
<td>1.25±0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>Mg²⁺ (1)</td>
<td>21.63±0.31</td>
<td>1.73</td>
</tr>
<tr>
<td>Co³⁺ (1)</td>
<td>30.25±0.41</td>
<td>2.42</td>
</tr>
<tr>
<td>Zn²⁺ (1)</td>
<td>17.25±0.26</td>
<td>1.38</td>
</tr>
</tbody>
</table>

* Mean value of three experiments ± SE.

Structure of the napA gene and deduced amino acid sequence of the NapA protein

The nucleotide sequence of the Morganella DNA insert of plasmid pPM9 was determined, and a computer analysis of possible coding regions allowed identification of a single ORF whose size was compatible with the results of SDS-PAGE analysis, and which was able to code for a polypeptide containing an amino acid sequence corresponding to the NH₂-terminal sequence of the NapA protein (Fig. 4). Since the insert of pPM9 terminated at a Sau3AI site located a short distance upstream of the start codon (Fig. 4), part of the insert of pPM2 was also sequenced to determine the structure of the 5'-flanking region of the napA gene.

The napA ORF has the potential to code for a polypeptide of 236 amino acids with a predicted molecular mass of 26686 Da. The deduced amino acid sequence of the NH₂-terminal region of the polypeptide showed features resembling those of prokaryotic signal sequences for protein export to the periplasmic space (Oliver, 1985) and, on the basis of NH₂-terminal sequencing data, it actually appeared to function as a signal sequence which is cleaved by signal peptidase after the alanine residue at position 23. A putative ribosome-binding site resembling those of E. coli is located upstream of the start codon.

All E. coli strains harbouring recombinant plasmids which included the napA ORF and flanking sequences showed strong pNPP-hydrolysing activity and were able to produce the NapA protein. On the other hand, strains harbouring recombinant plasmids which contained only a portion of the napA ORF showed pNPP-hydrolysing activity comparable to that of E. coli DH5α(pBluescript) and were not able to produce the NapA protein (Fig. 2). These data were in agreement with the hypothesis that the above ORF actually encoded the NapA protein.

The presence of a similar genomic region including the napA ORF was also analysed in the M. morganii strain ATCC 25830T by means of PCR using primers MMNAP-
The signal sequence of the NapA protein is underlined. The PCR regions. Number 1 represents the first base of the start codon downstream inverted repeat possibly functioning as a ORF. The putative ribosome-binding site is identified as NapA ORF and flanking regions. Number 1 corresponds to the residue numbers on the left correspond to the residue numbers of the proteins of Proteus mirabilis (Pmi napA) with those of other sequenced bacterial genomes. The hypothetical protein, homology is followed through different reading frames (indicated as 1°, 2°, and 3°, respectively) and frameshifts introduced to follow homology are indicated; X indicates a stop codon.

Fig. 5. Comparison of the nucleotide acid sequences of the napA gene of M. morganii (Mmo napA) with those of the hypothetical protein of E. coli (Eco ORF; EMBL accession number Z66592) and P. mirabilis (Pmi ORF; Charles et al., 1985; EMBL accession number M15877) showing sequence homology to NapA. Numbers on the left correspond to the residue numbers of the proteins. Identical residues are indicated by an asterisk; conservative amino acid substitutions are indicated by a colon. In the comparison between the NapA enzyme and the P. mirabilis hypothetical protein, homology is followed through different reading frames (indicated as 1°, 2°, and 3°, respectively) and frameshifts introduced to follow homology are indicated; X indicates a stop codon.

When the deduced amino acid sequence of the napA of M. morganii NapA enzyme was compared with all sequences present in the EMBL database (release 37) by means of the TFASTA program (Pearson, 1990), a high degree of homology was found with two hypothetical proteins of unknown function encoded by sequenced regions of the E. coli and Proteus mirabilis genomes respectively. The hypothetical E. coli NapA homologue is encoded by an ORF located 89 min in the tyrB–w.a intergenic region (EMBL accession number Z66592), and shows an overall degree of homology of 60.7% (rising to 90.7% when conservative amino acid substitutions are allowed for) with the Morganella NapA enzyme (Fig. 5). Interestingly, the opposite strand of this ORF entirely encompasses another ORF encoding a DNA-binding protein specific for the E. coli origin of replication (cited in EMBL entry Z5592). With regard to the P. mirabilis NapA homologue, it is apparently encoded by an ORF located 0.4 kb upstream of the chromosomal cat gene which is involved in chloramphenicol resistance (Charles et al., 1985). In this case, however, only part of the sequence is available (corresponding to amino acids 1–157 of the Morganella NapA protein) and the ORF encoding the NapA homologue is

Identification of putative NapA homologues on the basis of sequence comparison

Comparison of the deduced amino acid sequence of the NapA protein with those of other sequenced bacterial periplasmic phosphatases including the E. coli PhoA (Chang et al., 1986), UshA (Burns & Beacham, 1986a), CpdB (Liu et al., 1986), AppA (Pradel et al., 1990) and AppA (Dassa et al., 1990) enzymes, and the four known class A acid phosphatases (Groisman et al., 1992; Pond et al., 1989; Thaller et al., 1994; M. L. Riccio and others, unpublished, EMBL accession number X64820), did not reveal any significant homology.
prematurely interrupted by a termination codon (Fig. 5). However, since the homology can be followed in alternative reading frames (Fig. 5), and since a NapA-like phosphatase is actually present in _P. mirabilis_ (M. C. Thaller and others, unpublished), it seems more likely that the interruptions in the _P. mirabilis_ ORF are due to sequencing errors than to actual mutations that led to a silencing event. Considering the alignment that can be obtained after frameshifts have been introduced to follow homology, the _P. mirabilis_ NapA homologue shows, in the sequenced region, an overall degree of homology of 79.9% (rising to 95.6% when conservative amino acid substitutions are allowed for).

**DISCUSSION**

Phosphatase activities of enteric bacteria have been studied from several viewpoints, but current knowledge on these enzymes is still incomplete.

With regard to periplasmic acid phosphatases, several different enzymes have been described and characterized at the sequence level, and different molecular classes of these enzymes can be recognized on the basis of sequence data and functional properties. These include: (a) the class A acid phosphatases, which are EDTA-resistant non-specific acid phosphatases composed of low molecular mass polypeptides (~25–27 kDa), thus far described in _S. typhimurium_ (Groisman et al., 1992; Weppelman et al., 1977), _M. morganii_ (Thaller et al., 1994) and _P. stuartii_ (M. L. Riccio and others, unpublished, EMBL accession number X64820); (b) the Agp-AppA family of enzymes described in _E. coli_, which includes two enzymes with somewhat different properties [an acid glucose phosphatase (Agp) and an acid phosphatase with a very low pH optimum (AppA)] which are homologous at the sequence level and are probably derived from the same ancestor gene (Dassa _et al._, 1990); (c) the periplasmic UDP-sugar hydrolases, thus far characterized and sequenced in _E. coli_ and _S. typhimurium_ (Burns & Beacham, 1986a, b) (in the latter species the gene is silent), but probably present in several enteric bacteria (Neu, 1968); (d) the cyclic phosphodiesterases, thus far sequenced in _E. coli_ (Liu _et al._, 1986), but apparently present in several enteric bacteria (Neu, 1968).

Characterization and sequencing of the _M. morganii_ NapA acid phosphatase, previously identified as a minor phosphate-irrepressible acid phosphatase (Thaller _et al._, 1994), enabled its recognition as a new molecular species of bacterial periplasmic acid phosphatase. Since an enzyme with similar properties has been described in _S. typhimurium_ (Uerkvitz, 1988; Uerkvitz & Beck, 1981), it is reasonable to hypothesize that this enzyme represents a NapA homologue produced by _S. typhimurium_. Moreover, genes homologous to _napA_ are present in the _E. coli_ and _P. mirabilis_ genomes and, although their products have not been characterized, we have recently described in _E. coli_ a nonspecific acid phosphatase composed of a 27 kDa polypeptide component (Rossolini _et al._, 1994) which could correspond to the product of the _napA_ homologue found in this species, and we have also found a NapA-like protein in _P. mirabilis_ (M. C. Thaller and others, unpublished). The _Morganella_ NapA enzyme, therefore, appears to be a member of a family of conserved enzymes which we propose designating class B bacterial acid phosphatases, and which could be widespread among enteric bacteria. We are currently investigating the presence of NapA homologues in other enteric bacteria, and preliminary results indicate a widespread distribution of such enzymes among members of this family (M. C. Thaller and others, unpublished).

The class B bacterial acid phosphatases thus far characterized (i.e. the _Morganella_ NapA and the _Salmonella_ NapII enzymes) are nonspecific acid phosphatases composed of low molecular mass polypeptides similar to class A enzymes, but unlike the latter they apparently require a metal cofactor for activity and are more susceptible to phosphatase inhibition. Interestingly, these enzymes are also endowed with phosphotransferase activity, a property that could be relevant in cell physiology, especially for an enzyme located in the periplasmic space. The physiological role of these enzymes remains to be established. In _S. typhimurium_, the NapII enzyme has been considered as the major periplasmic 5'-nucleotidase (Uerkvitz & Beck, 1981). It would be interesting to confirm a similar role in other bacterial species and to investigate whether other functions are dependent on these class B enzymes.

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**REFERENCES**


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