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Structural Gene for the Phosphate-Repressible Phosphate-Binding Protein of *Escherichia coli* Has Its Own Promoter: Complete Nucleotide Sequence of the *phoS* Gene

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The complete nucleotide sequence of the *phoS* gene, the structural gene for the phosphate-repressible, periplasmic phosphate-binding protein *Escherichia coli* K-12, was determined. The phosphate-binding protein is synthesized in a precursor form which includes an additional N-terminal segment containing 25 amino acid residues, with the general characteristics of a signal sequence. The amino acid sequence derived from the nucleotide sequence shows the mature protein to be composed of 321 amino acids with a calculated molecular weight of 34,427. The *phoS* gene is not part of an operon and is transcribed counterclockwise with respect to the *E. coli* genetic map. A promoter region has been identified on the basis of homology with the consensus sequence of other *E. coli* promoter regions. However, an alternative promoter region has been identified on the basis of homology with the promoter regions of the *phoA* and *phoE* genes, the structural genes for alkaline phosphatase and outer-membrane pore protein e, respectively.

In *Escherichia coli* there are two major systems for phosphate uptake, the phosphate (inorganic) transport system and the phosphate-specific transport (Pst) system (26, 33). The latter is typical of a class of inducible high-affinity transport systems which are sensitive to osmotic shock and include periplasmic binding proteins (4, 17). The Pst system exerts negative control over alkaline phosphatase synthesis, unlike the phosphate (inorganic) transport system, which has no effect on alkaline phosphatase regulation (33). The Pst system itself is part of the phosphate regulon (32), in that depriving *E. coli* of phosphate results in its induction. Other genes which belong to the phosphate regulon include *phoA* and *phoE*, the structural genes for alkaline phosphatase and outer-membrane pore protein e, respectively (2).

The Pst system is coded for by genes located at 83 min on the *E. coli* genetic map (2), and four closely linked genes (*pstA*, *pstB*, *phoT*, and *phoS*) have been identified (5). The order of genes in this region of the *E. coli* genetic map is *phoU pstA* (*pstB phoT*) *phoS glmS uncC* (1, 5, 36). It was concluded that *phoU* (previously designated *phoT35*) was not likely to be part of the genes specifying the Pst system (36). The only gene product of the Pst system that has been identified is the phosphate-binding protein, coded for by the *phoS* gene (9). Cox et al. (5) proposed that the *pstA*, *pstB*, *phoT*, and *phoS* genes form an operon, with the *pstA* gene being promoter proximal, on the basis of polarity effects observed in studies of mutants affected in the Pst system. However, Amemura et al. (1) concluded from complementation studies with a plasmid carrying only the *phoS* gene that the *phoS* gene has its own promoter.

In the present work we determined the nucleotide sequence of the *phoS* gene, including the promoter region, and established the orientation of the *phoS* gene on the *E. coli* chromosome.

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MATERIALS AND METHODS

Bacterial strains and plasmids. All of the bacterial strains used were derived from *E. coli* K-12 and are described in Table 1. Plasmids used are described in Table 1, Fig. 1, and the text. Plasmid DNA was prepared as described by Downie et al. (7).

Genetic techniques. Transformations were carried out as described by Lederberg and Cohen (15).

Preparation of phosphate-binding protein. Phosphate-binding protein was prepared as described previously (9).

Amino acid sequencing. Automated amino acid sequencing was performed on a Beckman 890C Sequencer with a 1M Quadrol program. Residues were identified, after manual conversion to the phenylthiohydantoin derivatives, by high-pressure liquid chromatography on a Hewlett-Packard model 1084B instrument with an Altex 165 variable wavelength detector; the column was Zorbax ODS (Du Pont Co., Wilmington, Del.) with elution by sodium acetate-acetonitrile. Any ambiguity was resolved by amino acid analysis after hydrolysis in HCl-$SnCl_2$ (18).

Peptide mapping and amino acid analysis. Peptide mapping was done as previously described (9). Peptides were eluted from the paper, hydrolyzed for 22 h at 110°C in 6 M HCl, and analyzed on a Beckman 120C analyzer. The amino acid composition of purified phosphate-binding protein was determined by using a Beckman System 6300 high-performance analyzer (13).

Analysis of plasmid DNA. Plasmid DNA was digested by the appropriate restriction endonucleases, and the sizes of DNA fragments were determined as described previously (6). Physical maps of plasmids were deduced fom the electrophoretic patterns of digests and suitable double digests of plasmid DNA with various restriction enzymes.

DNA sequencing. Nucleotide sequences were determined by the method of Maxam and Gilbert (16). End-labeling of DNA was carried out with DNA polymerase and either [α-^{32}P]dATP or [α-^{32}P]dCTP (16).

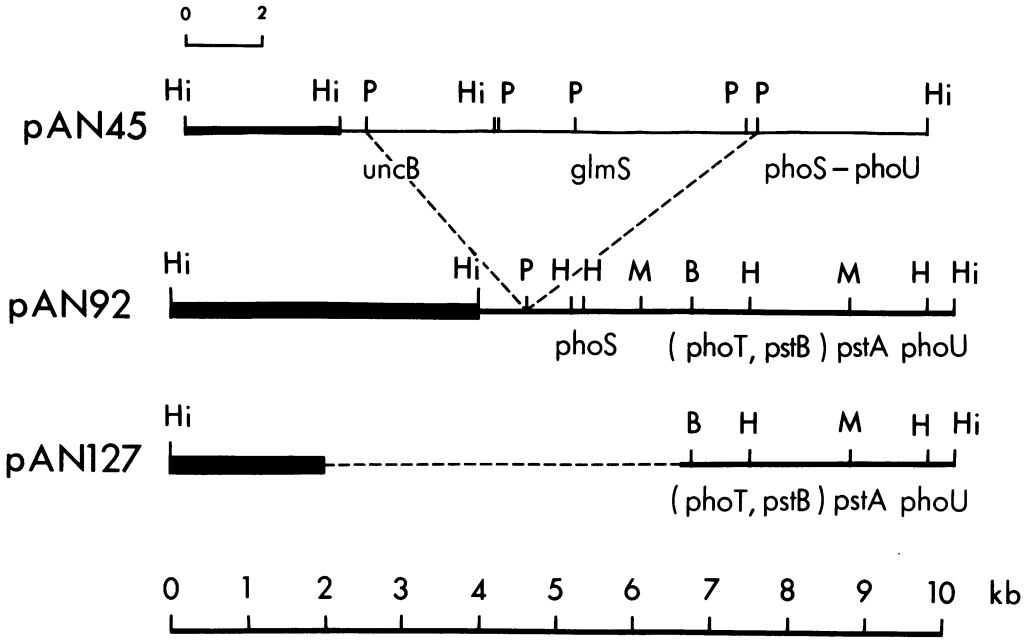

FIG. 1. Physical maps of plasmids pAN45, pAN92, and pAN127. Restriction endonuclease sites: B, *Bst*EII; H, *Hpa*I; Hi, *Hin*dIII; M, *Mlu*I; P, *Pst*I. Plasmid pAN92 was derived from plasmid pAN45 by deletion of the 10-kilobase *Pst*I fragment. The broken line shown in the restriction map for plasmid pAN127 indicates the extent of the deletion introduced during the construction of plasmid pAN127. The approximate locations of genes on the various plasmids are as shown. The scale used to represent plasmid pAN45 (upper-left portion of the figure) is twice that for plasmids pAN92 and pAN127. The order of genes shown left to right in the plasmids corresponds to the counterclockwise direction on the *E. coli* genetic map. kb, Kilobase.

Enzymes and chemicals. *E. coli* DNA polymerase was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Restriction endonucleases, [α-^{32}P]dATP, and [α-^{32}P]dCTP were obtained from the Radiochemical Centre (Amersham, England). All chemicals used were of the highest quality available.

RESULTS

N-terminal amino acid sequence of the phosphate-binding protein and amino acid analyses of some chymotryptic-tryptic peptides. Phosphate-binding protein was purified from the shock fluid of strain AB3311 as previously described (9). The N-terminal amino acid sequence of the purified phosphate-binding protein was determined by automated amino acid sequencing. The first twelve amino acids of the native protein were found to be:

1 5 10
Glu-Ala-Ser-Leu-Thr-Gly-Ala-Gly-Ala-Thr-Phe-Pro

Amino acid analysis of some chymotryptic-tryptic peptides, prepared as described previously (9), was also performed. The compositions of four chymotryptic-tryptic peptides (termed P1, P2, P3, and P4, respectively) were: Glx (Asx, Thr, Gly) Lys; (Asx, Ser, Glx, Pro, Ala, Val_2, Ile, Leu) Arg; (Glx, Val) Arg; (Ile_2, Leu, His) Lys. This information was subsequently used to determine the *phoS* reading frame (see below).

Restriction nuclease map and sequencing strategy. Plasmid pAN92 was derived from plasmid pAN45 (13) by deleting the 10-kilobase fragment between the *Pst*I restriction sites (Fig. 1). Plasmid pAN127 was constructed from plasmid pAN36 by the introduction of a large deletion (5). Earlier work in this laboratory (13) showed that plasmid pAN92 complemented mutations affecting the *pstA*, *pstB*, *phoT*, and *phoS* genes, whereas plasmid pAN127 also complemented mutations in the *pstA*, *pstB*, and *phoT* genes but not mutations affecting the *phoS* gene. It was concluded that plasmid pAN92 carried the *phoS* gene, whereas plasmid pAN127 did not. Restriction nuclease maps of plasmids pAN92 and pAN127 were constructed (Fig. 1) as outlined above. The region of chromosomal and vector DNA of plasmid pAN92 which is missing from plasmid pAN127 is indicated in Fig. 1 by the dotted line. This segment of DNA is likely to encompass the *phoS* gene. Furthermore, the region of DNA immediately to the left of the *Pst*I restriction site of pAN92 (Fig. 1) is part of the *uncB* gene (8). The *phoS* gene, therefore, must lie within the 2-kilobase *Pst*I-*Bst*EII fragment of plasmid pAN92 and may overlap the *Bst*EII site.

TABLE 1. *E. coli* strains and plasmids used

Strain or plasmid	Relevant genotype[a] and phenotype	Source or reference
AN1667	(pAN92)*ilvC argH entA srl::*Tn*10 recA*	Transformation of strain AN1664 (5) with pAN92
AN2098	(pAN127)*pstA2 argH pyrE entA srl::*Tn*10 recA*	Transformation of strain AN1685 (5) with pAN127
pAN92	Cm^r Tc^s *phoS*$^+$ *phoT*$^+$ *pstB*$^+$ *pstA*$^+$	(13)
pAN127	Cm^r Tc^s *phoT*$^+$ *pstB*$^+$ *pstA*$^+$	(5)

[a] Chromosomal gene nomenclature is according to Bachmann (2); plasmid gene nomenclature is according to Novick et al. (22).

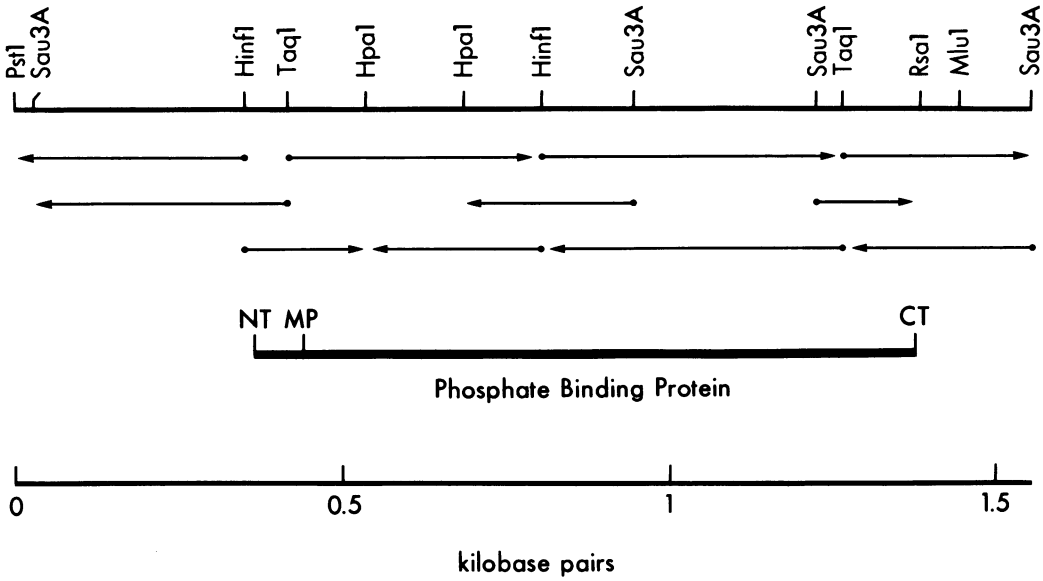

FIG. 2. Sequencing strategy. Restriction sites for the enzymes *Hin*fI, *Hpa*I, *Mlu*I, *Pst*I, *Rsa*I, *Sau*3A, and *Taq*I were determined as described in the text and were verified according to the established nucleotide sequence. With the exception of the *Mlu*I site, only the restriction sites used in the sequencing experiments are shown. The position of the ^{32}P-label at the 3′ end is indicated (●). The arrows show the restriction sites used to separate ^{32}P-labeled ends. The location of the structural gene for the phosphate-binding protein with respect to the region of DNA sequenced is shown. NT, N terminus of pre-phosphate-binding protein; MP, N terminus of mature phosphate-binding protein; CT, C terminus of phosphate-binding protein.

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5'TTCCTGTTATGTTTTTAATCAAACATCCTGCCAACTCCATGTGACAAACCGTCATCTTCGGCTACTTTTTCTCTGTCACAGAATGAAAATTTTTCTGTCATCTCTTCGTTATTAATGTTT-160

GTAATTGACTGAATATCAACGCTTATTTAAATCAGACTGAAGACTTTATCTCTCTGTCATAAAACTGTCATATTCCTTACATATAACTGTCACCTGTTTGTCCTATTTTGCTTCTCGTAG -40

                         oooo      MetLysValMetArgThrThrValAlaThrValValAlaAlaThrLeuSerMetSerAlaPheSerValPheAlaGluAla
CCAACAAACAATGCTTTATGAATCCTCCCAGGAGACATTATGAAAGTTATGCGTACCACCGTCGCAACTGTTGTCGCCGCGACCTTATCGATGAGTGCTTTCTCTGTGTTTGCAGAAGCA 81

SerLeuThrGlyAlaGlyAlaThrPheProAlaProValTyrAlaLysTrpAlaAspThrTyrGlnLysGluThrGlyAsnLysValAsnTyrGlnGlyIleGlySerSerGlyGlyVal
AGCCTGACAGGTGCAGGTGCAACCTTCCCTGCGCCGGTGTATGCCAAATGGGCTGACACTTACCAGAAAGAAACCGGTAATAAAGTTAACTACCAGGGTATCGGTTCTTCCGGTGGCGTA 201

LysGlnIleIleAlaAsnThrValAspPheGlyAlaSerAspAlaProLeuSerAspGluLysLeuAlaGlnGluGlyLeuPheGlnPheProThrValIleGlyGlyValValLeuAla
AAACAGATTATCGCTAATACCGTTGATTTTGGTGCCTCTGACGCGCCGCTGTCTGACGAAAAACTGGCTCAGGAAGGTCTGTTCCAGTTCCCGACCGTGATTGGCGGCGTGGTGCTGGCG 321

ValAsnIleProGlyLeuLysSerGlyGluLeuValLeuAspGlyLysThrLeuGlyAspIleTyrLeuGlyLysIleLysLysTrpAspAspGluAlaIleAlaLysLeuAsnProGly
GTTAACATTCCAGGGCTGAAGTCTGGCGAACTGGTGCTGGATGGTAAAACCCTCGGCGACATCTACCTGGGCAAAATCAAGAAGTGGGATGATGAAGCCATCGCCAAACTGAATCCGGGT 441

LeuLysLeuProSerGlnAsnIleAlaValValArgArgAlaAspGlySerGlyThrSerPheValPheThrSerTyrLeuAlaLysValAsnGluGluTrpLysAsnAsnValGlyThr
CTGAAACTGCCTTCACAAAACATTGCTGTAGTACGCCGCGCAGATGGCTCCGGGACTTCCTTCGTCTTCACCAGCTACCTGGCGAAAGTGAACGAAGAGTGGAAAAACAACGTTGGTACT 561

GlySerThrValLysTrpProIleGlyLeuGlyGlyLysGlyAsnAspGlyIleAlaAlaPheValGlnArgLeuProGlyAlaIleGlyTyrValGluTyrAlaTyrAlaLysGlnAsn
GGCTCTACCGTAAAATGGCCGATCGGTCTGGGCGGTAAAGGTAACGACGGTATCGCCGCGTTCGTTCAGCGTCTGCCGGGTGCAATTGGTTATGTTGAATATGCTTACGCGAAGCAGAAC 681

AsnLeuAlaTyrThrLysLeuIleSerAlaAspGlyLysProValSerProThrGluGluAsnPheAlaAsnAlaAlaLysGlyAlaAspTrpSerLysThrPheAlaGlnAspLeuThr
AACCTGGCGTACACCAAACTGATCTCCGCTGATGGTAAACCGGTTAGTCCGACCGAAGAAAACTTCGCTAATGCAGCAAAAGGTGCAGACTGGAGCAAAACCTTCGCTCAGGATCTGACC 801

AsnGlnLysGlyGluAspAlaTrpProIleThrSerThrThrPheIleLeuIleHisLysAspGlnLysLysProGluGlnGlyThrGluValLeuLysPhePheAspTrpAlaTyrLys
AACCAGAAAGGCGAAGATGCATGGCCTATTACCTCTACCACGTTCATTCTGATCCACAAAGATCAGAAGAAACCAGAACAAGGCACAGAAGTGCTGAAATTCTTCGACTGGGCGTACAAA 921

ThrGlyAlaLysGlnAlaAsnAspLeuAspTyrAlaSerLeuProAspSerValValGluGlnValArgAlaAlaTrpLysThrAsnIleLysAspSerSerGlyLysProLeuTyr *
ACCGGGGCTAAACAGGCGAACGACCTGGATTACGCCAGCCTGCCGGATAGTGTAGTTGAACAGGTTCGCGCTGCGTGGAAGACCAATATTAAAGACAGTAGCGGTAAGCCGCTGTACTAA1041

*
TAAAACTCCAGGCCGGGTACGGTGTTTTACGCCGCATCCGGCATTACAAAATGACTTTGTAAACGCGTTTAACTGAAGAGTAACTTATGGCTGCAACCAAGCCTGCTTTTAACCCACCGG1161

GTAAAAAGGGCGACATAATTTTCAGCGTGCTGGTAAAACTGGCGGCGCTGATTGTGC3'
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FIG. 3. DNA nucleotide sequence of the *phoS* gene, including possible promoter and terminator regions, and the deduced amino acid sequence. The nucleotides are numbered taking the position of the first nucleotide of the translational initiating codon (ATG) as +1. The amino acid sequence of the N terminus and the chymotryptic-tryptic peptides P1, P2, P3, and P4 of the phosphate-binding protein (see the text) are underlined. Nucleotides representing part of the potential ribosome-binding site are marked by an open circle. Asterisks denote the translational stop codons (TAA).

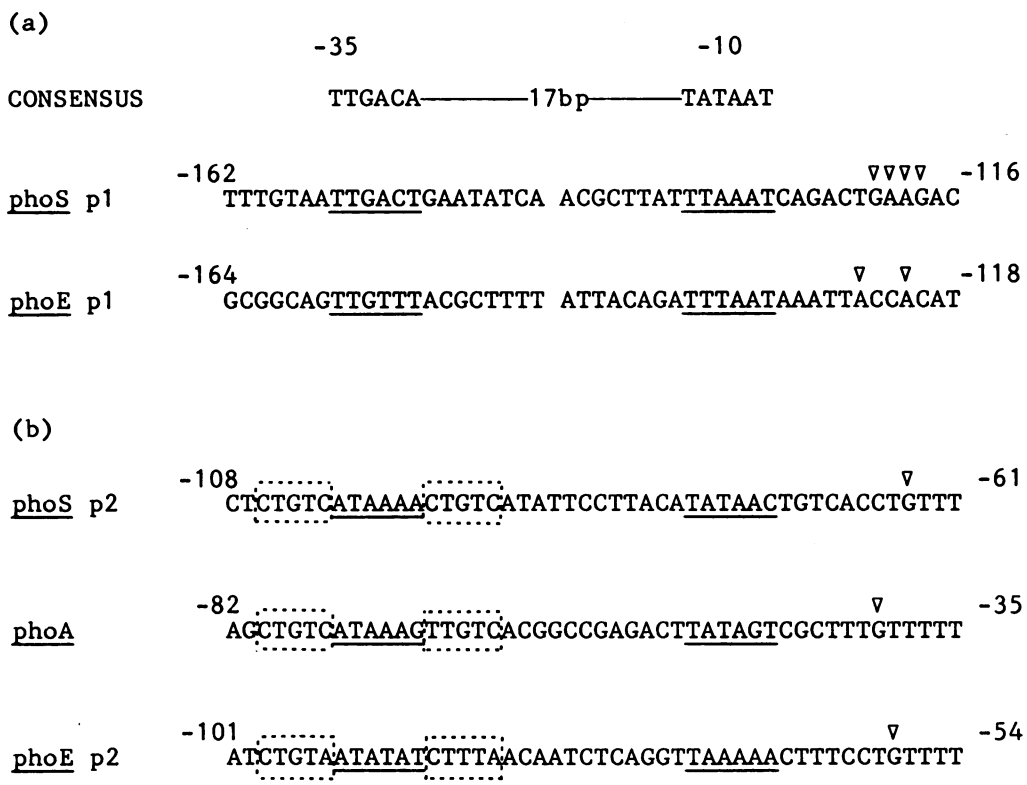

FIG. 4. Comparison of the nucleotide sequences of the potential promoter regions of *phoS*, *phoA*, and *phoE*. (a) Nucleotide sequences of the potential promoters *phoS* p1 and *phoE* p1 (the promoter furthest from the translational start site). (b) Nucleotide sequences of the *phoS* p2, *phoA*, and *phoE* p2 potential promoters. The nucleotide sequences of the potential promoter regions of *phoA* and *phoE* are here numbered in the same manner as *phoS* (Fig. 3). This numbering differs from that used in the original publications (14, 23). Nucleotide sequences showing homology to the consensus −35 and −10 regions are underlined. The pentanucleotide sequences on either side of the −35 region of *phoS* p2 and the corresponding nucleotides in the *phoA* p and *phoE* p2 are boxed. Possible start points of transcription are indicated by (▽). The nucleotide sequences of the *phoS* and *phoE* p1 potential promoters have been interrupted at an arbitrary point to produce the consensus 17-base-pair interval between the −10 and −35 regions.

The strategy used for sequencing the *Pst*I-*Bst*EII segment is shown in Fig. 2.

Nucleotide sequence of the *phoS* gene. The nucleotide sequence (Fig. 3) corresponding to the 12 N-terminal amino acids of the mature phosphate-binding protein was located on the 0.4-kilobase *Hin*fI fragment containing the two *Hpa*I sites (Fig. 2). An open reading frame extended from either side of this nucleotide sequence (Fig. 3). It is concluded that this open reading frame comprises the *phoS* gene coding for the pre-phosphate-binding protein. It was previously shown that the phosphate-binding protein has a precursor form (20). There are two possible translation initiation codons (ATG) in the reading frame preceding the region corresponding to the N-terminal end of the mature protein. The ATG codon at nucleotides 1 to 3 (see Fig. 3) is the most likely translational start site since it is located five nucleotides downstream from the last adenine residue of the nucleotide sequence AGGA, which forms part of the ribosome-binding site (28). Such spacing is consistent with all other analyzed procaryotic nucleotide sequences of the translation start region (28). There is only one open reading frame, extending to nucleotide 1038, and this is terminated by two nonsense codons (TAA) in tandem at nucleotides 1039 to 1044. This open reading frame codes for a protein of 346 amino acids with a molecular weight of 37,030.

The N-terminal glutamate residue of the mature phosphate-binding protein is coded for by the nucleotide triplet GAA at positions 76 to 78. The mature form of the phosphate-binding protein has 321 amino acids and a molecular weight of 34,427. The amino acid composition of the mature phosphate-binding protein derived from the nucleotide sequence in Fig. 3 closely resembles the amino acid composition of the purified phosphate-binding protein (Table 2). The derived amino acid sequence (Fig. 3) shows four chymotryptic-tryptic peptides corresponding to P1, P2, P3, and P4 (see above). This establishes that the open reading frame present in the nucleotide sequence is correct and codes for the phosphate-binding protein.

Nucleotide sequences adjacent to the *phoS* structural gene. No open reading frame was found in sequences extending 100 nucleotides beyond each end of the structural gene for the phosphate-binding protein. The DNA preceding the start point of translation of *phoS* at nucleotide 1 (Fig. 3) has sequences that could be involved in the interaction with RNA polymerase and which are known to be highly conserved in the promoters of *E. coli* genes (10, 27). Procaryotic promoters consist of a −35 region, involved in the recognition and binding of the σ factor of RNA polymerase holoenzyme, and a −10 region (or "Pribnow box"), which constitutes the binding site for the RNA polymerase core enzyme (25). There are five combinations of −35 and −10 regions from nucleotides −279 to −20 (Fig. 3). One of these, *phoS* promoter one (p1; Fig. 4), has the best homology with the consensus promoter sequence. *phoS* promoter two (p2; Fig.

4), in comparison with the consensus promoter sequence, has a highly conserved −10 region and a weakly conserved −35 region. A palindromic nucleotide sequence (CTGTC) is located on either side of the −35 region of *phoS* p2 (Fig. 4). *phoS* p2 has the best homology with the putative *phoA* and *phoE* promoters (14, 23). However, to establish the true functional promoter, S1 nuclease mapping (3) of the promoter region of the *phoS* gene must be performed.

An inverted repeat sequence, which can form the stable RNA hairpin structure (Fig. 5) thought to be involved in the termination of transcription (27), can be found at nucleotides 1053 to 1083 (Fig. 3). The inverted repeat sequence is guanine-cytosine rich and is followed by an adenine-thymine-rich region, characteristics which are shared by most known termination signals (27).

DISCUSSION

The *phoS* gene codes for a protein of 346 amino acids, which is the precursor form of the phosphate-binding protein. A precursor form of the phosphate-binding protein has been reported by Morita et al. (20). The mature phosphate-binding protein has 321 amino acids and a molecular weight of 34,427. The 25 additional amino acids present in the pre-phosphate-binding protein constitute a typical signal peptide (19), with a positively charged N terminus followed by a chain of 20 hydrophobic amino acid residues. The postulated N terminus of the pre-phosphate-binding protein has been confirmed by amino acid analysis of the purified protein (K. Magota et al., manuscript in preparation). The site of cleavage of the signal peptide to form the mature phosphate-binding protein lies on the carboxyl side of the alanine residue preceding the N-terminal glutamate of the mature phosphate-binding protein.

A potential ribosome-binding site consisting of the nucleotide sequence AGGA (28) is located five nucleotides before the translational start site. Within the nucleotide sequence preceding this ribosome-binding site, extending from nucleotides −279 to −20, two regions have been identified as likely functional promoters. One promoter region (*phoS* p1), consisting of the nucleotides TTAAAT (−10 region) and TTGACT (−35 region), shows very good homology with the consensus sequence for procaryotic promoters (10, 27). The second promoter (*phoS* p2) has a −35 region which has poor homology with the consensus promoter sequence and a −10 region which shows good homology with the consensus promoter sequence. The pentanucleotide sequence CTGTC

TABLE 2. Amino acid composition of the mature phosphate-binding protein

Amino acid	No. of residues	
	Deduced from DNA sequence	Amino acid analysis of purified phosphate-binding protein
Asn	17	37
Asp	20	
Thr	21	19.8
Ser	19	18.5
Gln	14	32.4
Glu	15	
Pro	15	15.6
Gly	34	33
Ala	35	34.4
Cys	0	0
Val	22	19.7
Met	0	0
Ile	17	13.9
Leu	24	23.9
Tyr	12	11.6
Phe	12	12.1
His	1	1.7
Lys	31	29.5
Arg	4	5.3
Trp	8	ND[a]

[a] ND, Not determined.

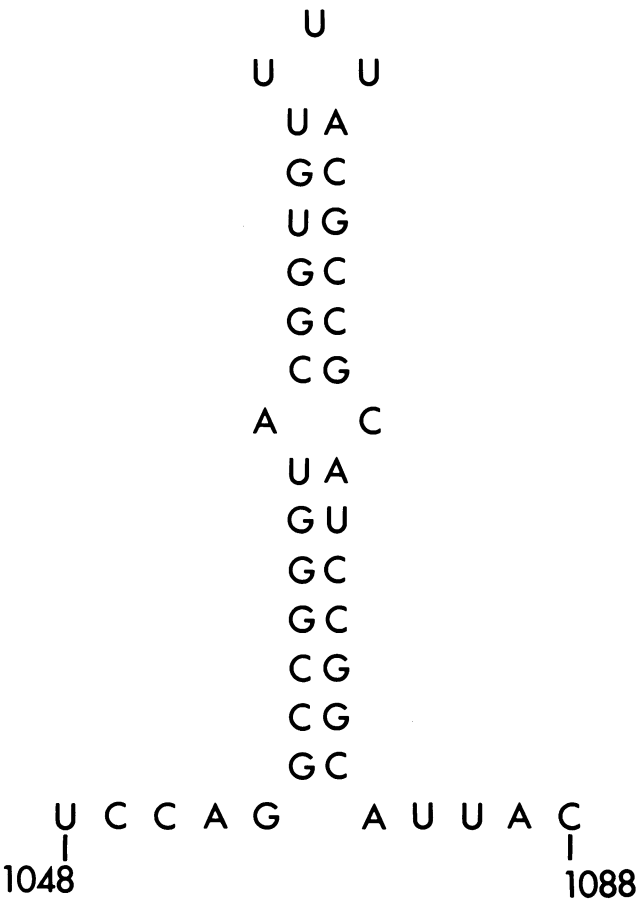

FIG. 5. Possible secondary structure of *phoS* mRNA at the 3′ end. Numbers of the corresponding nucleotides in the DNA sequence (Fig. 3) are indicated.

is located on either side of the −35 region of *phoS* p2 (see Fig. 4). *phoS* p2 has good homology with the putative promoter of the *phoA* gene, the structural gene for alkaline phosphatase (2). In the region preceding the *phoE* gene (23), two promoters can tentatively be identified, and we designated them *phoE* p1 and *phoE* p2, respectively (see Fig. 4). However, S1 nuclease mapping (3) is required before conclusions can be made as to the true functional promoter. In the DNA sequence beyond the end of the *phoS* gene, an inverted repeat sequence, capable of forming the stable RNA hairpin structure (see Fig. 5) thought to be involved in the termination of transcription (27), can be found at nucleotides 1053 to 1083.

The *phoS* gene is part of the phosphate regulon, which in *E. coli* K-12 comprises a set of more than 20 unlinked genes whose promoters can be regulated simultaneously by overlapping and separate controls (32). The *phoA* and *phoE* genes also belong to the phosphate regulon, and their regulation has much in common with that of the *phoS* gene. Thus, the *phoS*, *phoA*, and *phoE* genes are all induced by phosphate deprivation (12, 24, 31, 35) and are also subject to positive control by the *phoB* gene product (21, 30, 34) and to negative control by the *phoR* gene product (21, 30, 34). The homologous sequences present outside but close to the −35 and −10 regions of the *phoS*, *phoA*, and *phoE* genes could possibly form the basis of this regulation by *phoR*, *phoB*, or P_i deprivation. In this regard the observed pentanucleotide sequences on either side of the −35 region of *phoS* p2, as well as the corresponding sequences in the −35 regions of *phoA* p and *phoE* p2 (see Fig. 4), may be involved in positive regulation by the *phoB* gene product. It has been reported (11) that the two bacteriophage λ promoters, P_{RE} and P_I, subject to positive regulation by the *c*II protein, have a tetranucleotide sequence (TTGC) on either side of the −35 regions to which the *c*II protein binds. Furthermore, in the *gal* operon, the cAMP-cAMP receptor protein activator interacts at a target site (*cat*) located around position −35 (29).

The *phoS* gene was previously proposed (5) to be part of an operon containing the genes *pstA*, *pstB*, *phoT*, and *phoS*, with the *pstA* gene being promoter proximal. We showed in the present work that the *phoS* gene is not part of an operon and is transcribed in a counterclockwise direction, opposite to that proposed earlier (5). The polar nature of the *phoS* mutations reported earlier (5) could be explained by postulating that a gene product(s) of the putative Pst operon is involved in regulating the expression of the *phoS* gene in a positive manner. Plasmid pAN127, in comparison with plasmid pAN92, lacks not only the *phoS* gene but also part of another gene located on the DNA fragment between the *Mlu*I and *Bst*EII restriction sites in Fig. 1 (B. Surin, unpublished data). Polarity effects observed in genetic complementation tests of *phoS* mutants can thus be attributed either to mutations in a putative regulatory gene or to polar mutations in other genes which are located closer to the promoter for the Pst operon. This hypothesis would explain why plasmid pAN127 failed to complement *phoS* mutants (5), on the basis that it lacks a functional regulator gene. Work is currently underway in this laboratory to test this proposal.

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