Binding of a Protein-Tyrosine Phosphatase to DNA through Its Carboxy-Terminal Noncatalytic Domain

Vegesna Radha, Shubhangi Kamatkar, and Ghanshyam Swarup*

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

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ABSTRACT: The noncatalytic domain of a non-receptor-type protein-tyrosine phosphatase (the T-cell phosphatase or PTP-S) isolated from a rat spleen cDNA library shows homology with the basic domains of transcription factors Fos and Jun [Swarup, G., Kamatkar, S., Radha, V., & Rema, V. (1991) *FEBS Lett. 280,* 65–69]. We have expressed this phosphatase in *Escherichia coli* under the control of T7 promoter. The PTP-S gene product expressed in *E. coli* shows protein-tyrosine phosphatase activity and binds to DNA at pH 7.4 as determined by DNA affinity chromatography, Southwestern blotting, and gel retardation methods. The carboxy-terminal region of this phosphatase was fused with glutathione *S*-transferase by constructing expression vectors. Experiments using fusion proteins with glutathione *S*-transferase suggest that the carboxy-terminal 57 amino acids of PTP-S are sufficient for DNA binding. Deletion of the C-terminal 57 amino acids of PTP-S protein abolished its DNA binding property, as determined by Southwestern blotting, but not its enzymatic activity. This suggests that the C-terminal 57 amino acids are essential for the DNA binding function of this protein but not for its enzymatic activity. Another non-receptor-type protein-tyrosine phosphatase, PTP-1, when expressed in enzymatically active form in *E. coli* did not bind to DNA. These results suggest that a nontransmembrane protein-tyrosine phosphatase, PTP-S, binds to DNA in vitro through its carboxy-terminal noncatalytic region.

Phosphorylation of proteins at tyrosine residues is an important regulatory event in signal transduction pathways involved in mediating cell division, differentiation, and other cellular functions (Ullrich & Schlessinger, 1990; Cantley et al., 1991). Aberration in protein tyrosine phosphorylation can lead a cell to unrestrained growth and malignant phenotype, suggesting that the activity of protein-tyrosine kinases and the level of tyrosine phosphorylation of proteins in normal cells are tightly regulated (Hunter & Cooper, 1985). Protein-tyrosine phosphatases (PTPases)[1] are thought to play an important role in regulating the level of phosphotyrosine since most cellular responses require only a transient increase in protein tyrosine phosphorylation. Dephosphorylation of cdc2 protein kinase by cdc25 protein-tyrosine phosphatase is a critical event for the entry of the cells into mitosis (Gould & Nurse, 1989; Kumagai & Dunphy, 1991). Molecular characterization and the specific role of individual PTPases have received much less attention as compared to those of protein-tyrosine kinases. This was partly due to the belief that phosphatases simply reverse the effect of protein kinases by dephosphorylating substrates constitutively (Hunter, 1989). The availability of purified alkaline phosphatases, which can selectively dephosphorylate proteins phosphorylated at tyrosine (Swarup et al., 1981), proved to be a useful tool for in vitro experiments in the absence of purified PTPases.

Earlier studies on the search for PTPases established that these are specific enzymes, many of which could be specifically inhibited by low concentrations of zinc and orthovanadate (Brautigan et al., 1981; Foulkes et al., 1981; Swarup et al., 1982). Recent findings show that PTPases can be grouped into two classes. The receptor-type PTPases are represented by transmembrane proteins such as CD45 which possess an extracellular domain for ligand binding and generally two repeated phosphatase domains in the intracellular segment (Charbonneau et al., 1989; Kaplan et al., 1990; Krueger et al., 1990). Several receptor-type PTPases have been discovered which may be regulated by as yet unidentified specific ligands (Fischer et al., 1991; Saito & Streuli, 1991).

The non-receptor-type PTPases possess a single catalytic domain of about 230 amino acids which is highly conserved (Brown Shimer et al., 1990; Chernoff et al., 1990; Cool et al., 1989; Guan et al., 1990; Swarup et al., 1991; Champion Arnaud et al., 1991). This homology of the catalytic domain is also seen with the receptor-type PTPases. In addition to the catalytic domain, the non-receptor-type PTPases possess over 100 amino acids in the noncatalytic domain usually located at the carboxy-terminal end. Human placental PTPase 1B, which was purified from cytosolic as well as membrane fractions, showed only 321 amino acids although the cDNA coding for it contained an additional 114 amino acids at the carboxy-terminal end (Charbonneau et al., 1989; Chernoff et al., 1990; Guan et al., 1990). The noncatalytic regions of different non-receptor-type PTPases such as placental PTPase 1B and human T-cell PTPase show very little homology (Fischer et al., 1991). The function of the noncatalytic region of the protein may be specific for each PTPase (Cool et al., 1990).

A cDNA clone coding for a polypeptide of 415 amino acids showing 65% homology with PTP-1B was isolated from a human T-cell cDNA library (Cool et al., 1989). Two different forms of this PTPase, which arise due to alternative splicing, were subsequently isolated which differ in the carboxy-terminal region (Swarup et al., 1991; Champion-Arnaud et al., 1991; Mosinger et al., 1992). Within the noncatalytic regions of human T-cell PTPase and its rat homologue PTP-S, there is a region rich in basic amino acids which shows homology with the basic domains of the transcription factors Fos and Jun (Swarup et al., 1991). This basic region is not present in human placental PTPase 1B or its rat homologue PTP-1.

* Address correspondence to this author. Telephone: +91-842-852241. Telex: 425-7046 CCMB IN. Fax: +91-842-851195.

[1] Abbreviations: PTPase, protein-tyrosine phosphatase; IPTG, isopropyl β-D-thiogalactoside; GST, glutathione *S*-transferase; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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Strong conservation of clusters of basic amino acids in the noncatalytic regions of human T-cell PTPase and rat PTP-S suggests that these residues have an important function. Here we report that the bacterially expressed PTP-S protein binds to DNA and the DNA binding function is localized in the noncatalytic carboxy-terminal region.

MATERIALS AND METHODS

Construction of the Vector Expressing Enzymatically Active PTP-S Gene Product. A 1.3-kb *Hin*dIII fragment containing the entire coding region of PTP-S (Swarup et al., 1991) was cloned in the *Hin*dIII site of pUC-19. From this construct, a *Bam*HI fragment encompassing the entire coding region of PTP-S was isolated and cloned in the *Bam*HI site of the expression vector pET-3C (Rosenberg et al., 1987). The orientation of the cDNA insert was determined by restriction analysis. This construct named pET-PTP expressed a full-length PTP-S protein in the *Escherichia coli* host strain BL-21 Lys S. This protein contains 29 extra amino acids, 12 of which are from the vector itself (gene 10 protein of phage T7), and the remaining amino acids are contributed by the multiple cloning site region of pUC-19 and the 5′-untranslated region of PTP-S cDNA.

Standard molecular cloning procedures (e.g., restriction digestions, ligations, transformation, colony hybridization, electroelution, etc.) used here were carried out essentially according to published procedures (Sambrook et al., 1989).

Construction of the Vector for Expressing Truncated PTP-S. The plasmid carrying the PTP-S cDNA was digested with *Sma*I followed by partial digestion with *Hin*cII. From this digest, a 0.95-kb fragment carrying 942 base pairs of PTP-S cDNA was isolated by electroelution. This fragment was ligated with the *Bam*HI-digested end-filled pET-3c (Rosenberg et al., 1987). Positive clones were identified by colony hybridization, and the orientation of the insert was determined by restriction analysis. This construct, pET-PTPΔ, expressed a truncated PTP-S protein lacking the carboxy-terminal 57 amino acids, in *E. coli* host strain BL-21 Lys S upon induction with IPTG. This truncated PTP-S protein was enzymatically active and showed an apparent molecular weight of about 43 000.

Construction of the Vector Expressing PTP-1. A 2.6-kb *Nco*I fragment of rat PTP-1 cDNA (Guan et al., 1990) containing the entire coding region was cloned in the *Nco*I site of expression vector pKK-233-2 (Amann & Brosius, 1985). In this vector, the expression of the gene is under the control of the inducible *trc* promoter. After screening by colony hybridization, the orientation of the insert in positive clones was determined by restriction analysis. This vector, pKK-PTP-1, upon induction with IPTG produced a protein of about 50 kDa, as expected, in the *E. coli* host strain JM 109. The PTP-1 protein expressed by cells carrying this vector contains 432 amino acids of PTP-1 and no extra amino acids.

Construction of Vectors Expressing Fusion Proteins. In order to raise antibodies against the noncatalytic domain of PTP-S, a smaller clone of PTP-S (clone 2, nucleotides 750–1287) encoding amino acids 242–363 (Swarup et al., 1991) was cloned in the *Eco*RI site of vector pGEX-3x (Smith & Johnson, 1988). After screening by colony hybridization, the orientation of the insert was determined by restriction analysis. This construct, pGEX-NC-1, expresses glutathione *S*-transferase (GST) fused with the noncatalytic domain of PTP-S under the control of the hybrid *tac* promoter, giving a fusion protein of 41 kDa.

Another fusion protein with GST was made by cloning the *Hin*cII–*Eco*RI fragment (nucleotides 943–1495) of PTP-S, after end-filling, in the *Sma*I site of pGEX-2T (Smith & Johnson, 1988). After screening by colony hybridization, the orientation of the insert was determined by restriction analysis. This construct, pGEX-NC-2, gave a 34-kDa fusion protein with GST containing the carboxy-terminal 57 amino acids of PTP-S.

A *Pst*I fragment (nucleotides 662–1144; amino acids 212–363) of PTP-S was cloned into the *Pst*I site of pUEX-2 (Bressan & Stanely, 1987). This construct, named pUEX-NC, expressed a fusion protein which was used for affinity purifiction of antibodies directed against the noncatalytic domain of PTP-S.

Preparation of Antibodies. Antibodies against the noncatalytic domain of PTP-S were raised by immunizing rabbits with the 41-kDa fusion protein expressed by the pGEX-NC-1 vector. The fusion protein band was cut, homogenized in phosphate-buffered saline, and passed through a 21-gauge needle. White New Zealand rabbits were injected with about 0.5 mg of this protein (along with polyacrylamide) at 4–5-week interval. Four days after the third injection, 30 mL of blood was collected.

Affinity Purification of Antibodies. Affinity purification of the antibodies directed against the noncatalytic domain of PTP-S was carried out using the fusion protein expressed by the pUEX-NC vector essentially as described (Sambrook et al., 1989). This fusion protein was transferred electrophoretically to Immunobilon-P membranes, incubated with 0.2 mL of antiserum in 5 mL of TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween-20). After the membranes were washed, the bound antibody was eluted with 200 mM glycine hydrochloride buffer, pH 2.8.

Immunoblotting. Proteins were fractionated by SDS–polyacrylamide gel electrophoresis and transferred electrophoretically to Immobilon-P membranes. The resulting blot was incubated with 1% bovine serum albumin in TBST for 1 h and then with affinity-purified antibody in TBST for 2–3 h. After being washed with TBST several times, the blot was incubated with alkaline phosphatase conjugated second antibody for 1 h. After the blot was washed with TBST 3–4 times, color was developed using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Phosphatase Assay. Protein-tyrosine phosphatase activity was assayed by using ^{32}P-labeled poly(Glu^4,Tyr^1) as substrate as described by us previously (Swarup & Subrahmanyam, 1989).

DNA–Sepharose Chromatography. E. coli cells carrying the pET-PTP expression vector were induced for 3 h with 1 mM IPTG in the midlog phase. Cells from 1 mL of culture were pelleted and incubated with 0.5 mL of extraction buffer (50 mM Tris-HCl, pH 7.0, 10 mM EDTA, 0.025% 2-mercaptoethanol, 0.5 mg/mL lysozyme, and 1% Triton X-100) containing 0.2 M NaCl for 60 min at 4 °C. This suspension was cleared by centrifuging for 5 min at 15000*g*. The extract was diluted with binding buffer (20 mM Tris-HCl, pH 7.4, 1% NP-40, 40 mM NaCl, 1 mM EDTA, and 0.025% 2-mercaptoethanol) without salt (Bister et al., 1987) to adjust the concentration of NaCl to 40 mM. DNA–Sepharose was prepared by coupling native, double-stranded calf thymus DNA with cyanogen bromide activated Sepharose 4B (Sorger et al., 1989). The DNA–Sepharose column (2 × 0.7 cm; bed volume 0.8 mL) was equilibrated at 4 °C with binding buffer containing 40 mM NaCl. After the column was loaded with 0.2 mL of diluted extract, the column was washed with binding

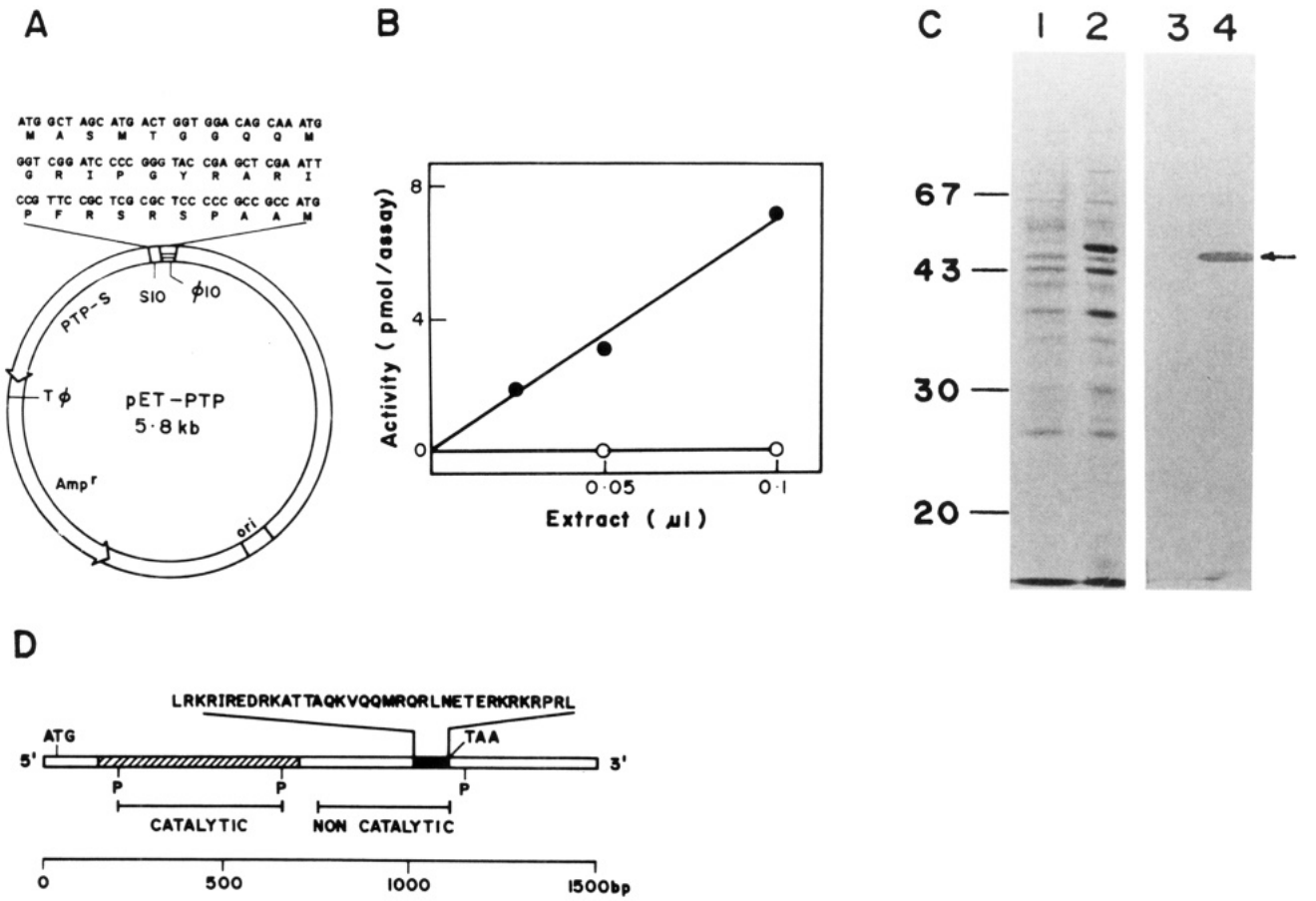

FIGURE 1: Expression of the PTP-S gene product in *E. coli*. (A) Full-length cDNA for PTP-S was cloned behind the T7 promoter by constructing the vector pET-PTP. This construct contains 29 additional amino acids (shown here) before the initiator methionine of PTP-S. (B) PTPase activity of *E. coli* cells expressing the PTP-S gene product. Extracts from cells containing cDNA in the sense orientation (solid circles) or in the antisense orientation (open circles) were used for assay. (C) Identification of the PTP-S gene product expressed in *E. coli* by SDS–PAGE (lanes 1 and 2) and immunoblotting using antibody directed against the noncatalytic domain (lanes 3 and 4). Lanes 1 and 3, control cell lysate; lanes 2 and 4, lysates from bacteria expressing the PTP-S gene product. (D) Maps of PTP-S cDNA showing the catalytic (hatched) and noncatalytic domains. The solid black segment denotes the basic region, the amino acid sequence of which is shown. P, *Pst*I site.

buffer (3 mL). Elution was carried out with 1-mL aliquots of binding buffer containing 0.1, 0.2, 0.4, 0.6, and 1.0 M NaCl. During assay of the fractions for PTPase activity, the salt concentration was equalized. For immunoblotting, 0.1-mL aliquots of fractons were run on 10% SDS–PAGE.

DNA Binding Studies Using Southwestern Blotting. Proteins were fractionated by SDS–PAGE and transferred electrophoretically to Immobilon-P membranes. This blot was probed with ^{32}P-labeled DNA essentially as described (Bowen et al., 1980). The blot was incubated for 18–20 h in buffer A containing 10 mM sodium phosphate, pH 7.4, 1 mM EDTA, 150 mM NaCl, 1 mM dithiothreitol, and 0.2% BSA. This blot was then probed with labeled DNA (4 ng/mL; 5×10^4 cpm/ng) in buffer A without BSA at room temperature for 1 h. It was then washed 4–5 times with buffer A without BSA and exposed for autoradiography.

*Gel Retardation Assay. Hin*fI-digested pBR322 was labeled with ^{32}P by end-filling. The proteins expressed by the vectors pET-PTP and pGEX-NC-1 were purified by electroelution from acrylamide gels, precipitated with acetone, and dissolved in water. These proteins were incubated with ^{32}P-labeled DNA (1 ng, 16 000 cpm) in 40 mM Tris–acetate, pH 7.4, and 1 mM EDTA at room temperature for 20 min. After incubation, xylene cyanol and glycerol in Tris–acetate buffer were added. Electrophoresis of these samples was done in a 4% polyacrylamide gel in 40 mM Tris–acetate buffer containing 1 mM EDTA as described by Fried and Crothers (1981). The gel was dried before autoradiography.

RESULTS

Expression of the Enzymatically Active PTP-S Gene Product in Bacteria. The amino acid sequence of PTP-S derived from cDNA showed stretches of basic amino acids in the noncatalytic carboxy-terminal domain (Figure 1D). This basic domain showed homology with the basic domains of transcription factors Fos and Jun (Swarup et al., 1991). Clusters of basic amino acids are present in many DNA binding proteins, and these basic regions are involved in the binding of these proteins to DNA (Busch & Sassone-Corsi, 1990). In order to explore the possibility that the PTPase coded by PTP-S cDNA may have DNA binding properties, this protein was expressed in *E. coli* under the control of the T7 promoter by constructing an expression vector, pET-PTP (Figure 1A). Extracts prepared from *E. coli* cells (induced with IPTG for 3 h) expressing the PTP-S gene product showed a high level of PTPase activity as determined by the dephosphorylation of ^{32}P-labeled poly(Glu^4, Tyr^1), a synthetic substrate. Control extracts prepared from cells carrying PTP-S cDNA in the antisense orientation did not show any detectable PTPase activity (Figure 1B). This expression vector, pET-PTP, produced a 47-kDa protein which was recognized by the antibody directed against the noncatalytic domain of the PTP-S gene product (Figure 1C). Rabbit antisera and affinity-purified antibodies which specifically recognize the noncat-

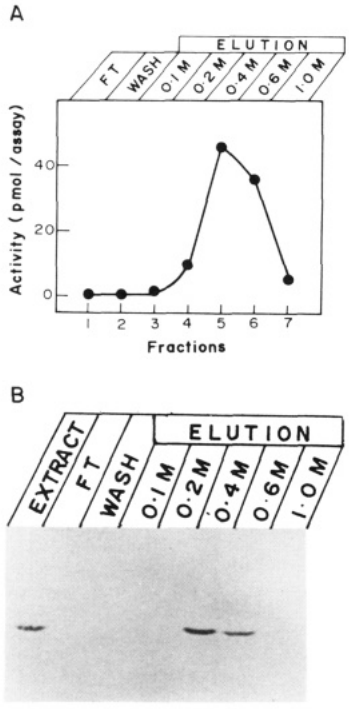

FIGURE 2: DNA binding assay of the bacterially expressed PTP-S gene product. (A) DNA–Sepharose chromatography of the extract from *E. coli* cells expressing the PTP-S gene product. Fractions including flowthrough (FT) and wash were assayed for PTPase activity. (B) Immunoblot analysis of fractions (0.1-mL aliquot) from DNA–Sepharose chromatography.

alytic domain were prepared as described under Materials and Methods. Figure 1C also shows that the antibody against the noncatalytic region is quite specific and does not show significant reaction with any *E. coli* protein except the 47-kDa polypeptide which is the product of the PTP-S gene.

The PTP-S gene product expressed in *E. coli* was partially characterized with respect to its enzymatic properties using ^{32}P-labeled poly(Glu^4, Tyr^1) as substrate. It was not inhibited by EDTA or mercaptoethanol; the extracts prepared in the presence of mercaptoethanol showed somewhat higher activity. It was inhibited by orthovanadate at micromolar concentrations. Its enzyme activity was stimulated by NaCl; removal of NaCl by dialysis resulted in nearly complete loss of activity which was regained by the addition of NaCl.

Bacterially Expressed PTP-S Gene Product Binds to DNA. Bacterially expressed PTP-S was used for studying the probable DNA binding function of this protein. Extraction of this PTPase from *E. coli* cells carrying the expression vector pET-PTP required 0.2–0.4 M NaCl in addition to 1% Triton X-100. *E. coli* extract containing the PTP-S gene product was chromatographed on a DNA–Sepharose column (native, double-stranded calf thymus DNA bound covalently to Sepharose 4B) after adjustment of the concentration to 40 mM NaCl and to pH 7.4. The PTPase activity was retained by this column and could be eluted, after washing, with increasing concentrations of salt (Figure 2A). Fractions from this column were analyzed by immunoblotting in order to confirm that the PTP-S gene product was bound to DNA–Sepharose (Figure 2B). When in a similar experiment Sepharose 4B was used instead of DNA–Sepharose, no PTPase activity was retained by the matrix (figure not shown). This suggests that the PTPase is retained by DNA–Sepharose due to interaction with DNA. DNA affinity chromatography has been used earlier to study the DNA binding properties of several proteins including the *c-myc* gene product and p53 (Bister et al., 1987; Steinmeyer & Deppert, 1988).

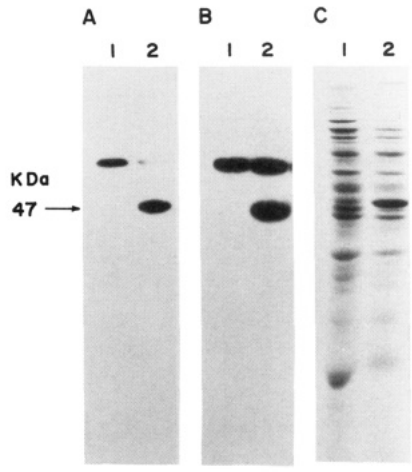

FIGURE 3: Binding of double-stranded (A) and single-stranded DNA (B) to the PTP-S gene product made in *E. coli*. Samples were analyzed by SDS–PAGE, transferred to Immobilon-P, and incubated with labeled rat genomic DNA. The DNA was labeled by nick-translation. Single-stranded DNA was prepared by alkali-denaturation of DNA. Lane 1, lysate from *E. coli* cells carrying the control pET vector; lane 2, lysate from cells producing the PTP-S gene product (pET-PTP vector). (C) Coomassie blue staining of *E. coli* lysates analyzed by SDS–PAGE. Lanes are the same as in (A) and (B).

We have also used the Southwestern blotting method to examine the ability of bacterially expressed PTP-S gene product to bind to double-stranded and single-stranded DNA. *E. coli* cell lysates from bacteria carrying pET-PTP or control plasmid were resolved by SDS–PAGE and transferred electrophoretically to Immobilon-P membranes. This blot was then incubated with nick-translated rat genomic DNA in phosphate buffer, pH 7.4, containing 150 mM NaCl, as described under Materials and Methods. As shown in Figure 3, both double-stranded as well as single-stranded DNA was bound to the PTP-S gene product of 47 kDa. The binding of DNA was dependent on pH. The binding of double-stranded DNA at pH 6 was much stronger than at pH 7 (figure not shown).

Localization of the DNA Binding Function in the Carboxy-Terminal Noncatalytic Domain. The DNA binding function of many proteins has been localized to a small portion of the molecule (Mitchel & Tjian, 1989). Since the carboxy-terminal noncatalytic domain of PTP-S is rich in basic amino acids and shows homology with the basic domains of DNA binding proteins, the possibility that this domain alone may be sufficient for DNA binding was examined. The carboxy-terminal 122 amino acids or 57 amino acids of PTP-S were fused at the carboxy-terminal end of a soluble cytoplasmic protein, glutathione *S*-transferase (GST), by constructing appropriate expression vectors, as described under Materials and Methods. GST is a protein of 27.5 kDa, and fusion with the carboxy-terminal 122 amino acids of PTP-S produced a protein of 41 kDa (Figure 4, lane 2); fusion of GST with 57 amino acids of PTP-S produced a protein of 34 kDa (Figure 4, lane 3). Extraction of GST from *E. coli* cells could be carried out in a buffer containing 1% Triton-100, but the extraction of the 41-kDa fusion protein required salt (0.6 M NaCl) in addition to the detergent (Figure 5). Extracts containing the 41-kDa fusion protein were prepared and chromatographed on a DNA–Sepharose column after the NaCl concentration was adjusted to 40 mM by dilution. After the column was washed, elution was carried out with increasing salt concentrations. All fractions, wash and flowthrough, from the column were examined for the presence of the 41-kDa fusion protein by immunoblotting (Figure 6A). It was observed that this fusion

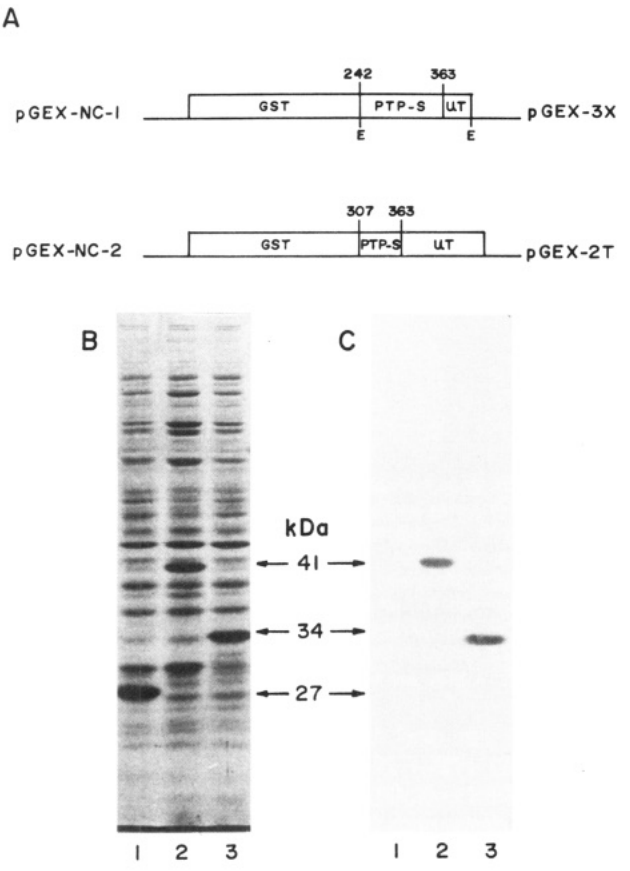

FIGURE 4: Bacterial fusion protein constructs and the GST–PTP fusion proteins. (A) The vectors pGEX-NC-1 and pGEX-NC-2 were constructed as described under Materials and Methods. The numbers (e.g., 307 and 363) indicate amino acid numbers of PTP-S. P, *Pst*I site; B, *Bam*HI site; E, *Eco*RI site; UT, untranslated region. (B) Coomassie blue staining of *E. coli* lysates analyzed by SDS–PAGE. Lane 1, lysate from bacteria producing the GST protein; lane 2, lysate from bacteria producing the 41-kDa GST–PTP fusion protein (by the pGEX-NC-1 vector); lane 3, lysate from bacteria carrying the pGEX-NC-2 vector producing the 34-kDa fusion protein. (C) Immunoblot using affinity-purified antibody directed against the noncatalytic domain of PTP-S. Lanes are the same as in (B).

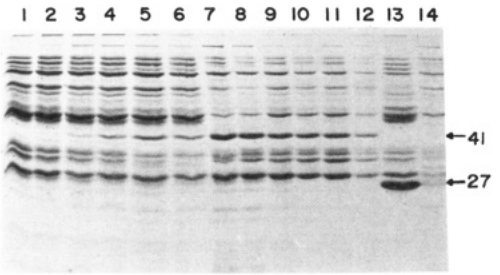

FIGURE 5: Extraction of the GST–PTP fusion protein of 41 kDa with salt. Extractions were carried out with the indicated concentration of salt, as described under Materials and Methods. Extracts were analyzed by SDS–PAGE. Lanes 1–6 and 13 are supernatants; lanes 7–12 and 14 are the pellet after extraction. Lanes 1–12, *E. coli* cells carrying the pGEX-NC-1 vector expressing the GST–PTP fusion protein of 41 kDa; lanes 13 and 14, cells carrying the control pGEX vector expressing the GST protein. Lanes 1, 7, 13, and 14, no NaCl; lanes 2 and 8, 0.1 M NaCl; lanes 3 and 9, 0.2 M NaCl; lanes 4 and 10, 0.4 M NaCl; lanes 5 and 11, 0.6 M NaCl; lanes 6 and 12, 1 M NaCl.

protein was retained by the DNA–Sepharose column and was eluted with 0.4–0.6 M salt in the elution buffer. The native GST protein of 27.5 kDa did not bind to DNA–Sepharose and appeared in the flowthrough or washings (Figure 6B). The 34-kDa fusion protein also showed binding to DNA–Sepharose and could be eluted with increasing salt concentrations (figure not shown). These results suggest that the carboxy-terminal noncatalytic region of PTP-S is sufficient for the DNA binding function of this protein.

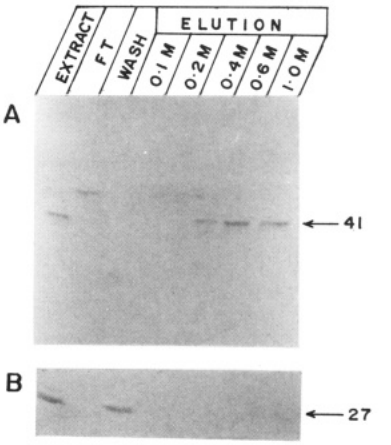

FIGURE 6: DNA binding assay of GST and GST–PTP fusion proteins. (A) DNA–Sepharose chromatography of the extract from *E. coli* cells expressing the fusion protein of GST with the carboxy-terminal 122 amino acids of PTP-S. Column fractions (0.1-mL aliquot) were analyzed by immunoblotting. The band seen in lane FT is perhaps due to some cross-reactivity of the antibody with an *E. coli* protein. (B) DNA–Sepharose chromatography of the GST protein. Fractions were analyzed by SDS–PAGE and stained with Coomassie blue.

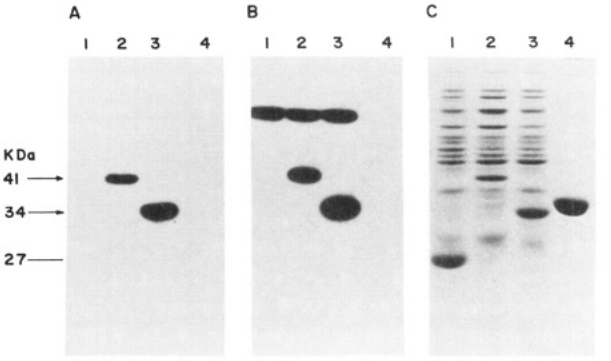

FIGURE 7: Binding of double-stranded (A) and single-stranded (B) DNA to fusion proteins of GST with carboxy-terminal regions of PTP-S. Samples were analyzed by SDS–PAGE, transferred to Immobilon-P, and incubated with labeled rat genomic DNA as described under Materials and Methods and in the legend to Figure 3. Lane 1, lysate from *E. coli* cells expressing the GST protein; lane 2, lysate from bacteria expressing the GST–PTP fusion protein of 41 kDa whose synthesis is directed by the vector pGEX-NC-1; lane 3, lysate from bacteria carrying the pGEX-NC-2 vector producing the 34-kDA fusion protein; lane 4, lactate dehydrogenase, 5 μg (Boehringer). (C) Coomassie blue staining of *E. coli* lysates analyzed by SDS–PAGE. Lanes are the same as in (A) and (B).

The DNA binding ability of these fusion proteins of 41 and 34 kDa was also analyzed by the Southwestern blotting method. As shown in Figure 7, these fusion proteins bind to double-stranded as well as single-stranded rat genomic DNA. The control GST protein does not bind to either single-stranded or double-stranded DNA (Figure 7, lane 1). These observations provide further evidence for the suggestion that 57 amino acids at the carboxy-terminal end of PTP-S are sufficient for DNA binding.

The PTP-S gene product produced in *E. coli* is slightly modified since it contains 29 additional amino acids at the amino-terminal end. Some of these additional amino acids are positively charged and, therefore, could possibly contribute toward the observed DNA binding properties of the 47-kDa PTP-S gene product. Experiments with the GST fusion protein suggest that the carboxy-terminal 57 amino acids of this protein are sufficient for DNA binding.

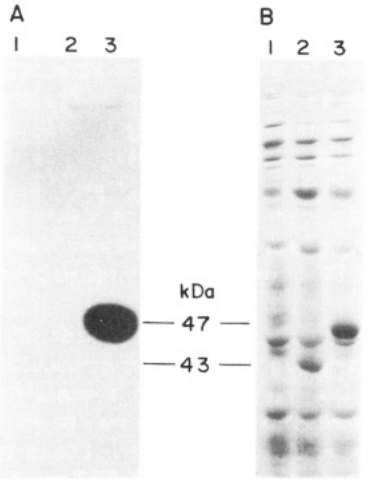

FIGURE 8: Truncated form of PTP-S does not bind to DNA. Expression of the truncated form of PTP-S was achieved by inducing the *E. coli* cells carrying the vector pET-PTPΔ with IPTG for 4 h. Construction of this vector is described under Materials and Methods. (A) Binding of DNA to full-length and truncated PTP-S proteins analyzed by Southwestern blotting using nick-translated plasmid pGEM-3Z DNA as probe. Lane 1, control cell lysate; lane 2, lysate from cells expressing the truncated form of PTP-S; lane 3, lysate from cells expressing full-length PTP-S. (B) Coomassie blue stained gel of samples used for blotting in (A).

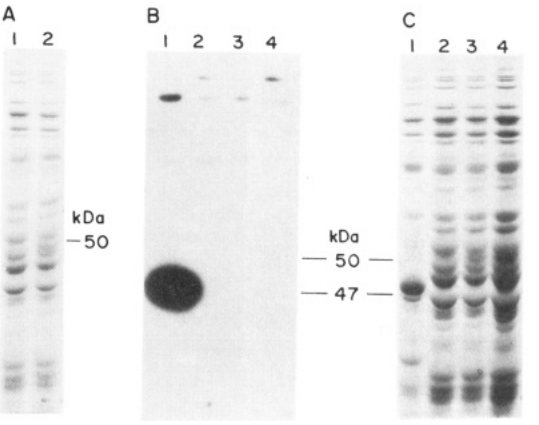

FIGURE 9: PTP-1 does not bind to DNA. (A) PTP-1 was expressed in *E. coli* by inducing the cells carrying the pKK-PTP-1 vector for 4 h with IPTG. The cell lysates were analyzed by polyacrylamide gel electrophoresis in the presence of SDS. Lane 1, control cell lysate; lane 2, lysate from cells carrying the vector pKK-PTP-1. (B) Binding of DNA to PTP-1 and PTP-S analyzed by Southwestern blotting using nick-translated plasmid pGEM-3Z DNA as probe. Lane 1, lysate from bacteria expressing PTP-S carrying the vector pET-PTP-S; lane 2, control cell lysate; lane 3, lysate from bacteria expressing PTP-1; lane 4, same lysate as in lane 3 but twice the amount. (C) Coomassie blue stained gel of samples used for blotting in (B). The gel was overrun in order to get good separation of the PTP-1 protein of 50 kDa.

Under the conditions of our experiments, lactate dehydrogenase, which is known to bind to single-stranded DNA (Williams et al., 1985), did not bind to single-stranded or double stranded DNA (Figure 7, lane 4). This suggests that the binding of DNA in blot binding experiments to PTP-S is much stronger than to lactate dehydrogenase.

A vector, pET-PTPΔ, was constructed for the expression of truncated PTP-S protein as described under Materials and Methods. This vector upon induction with IPTG directed the synthesis of a truncated protein of 43 kDa which was expressed at fairly high levels and showed PTPase activity, but it did not bind to DNA as determined by Southwestern blotting (Figure 8). This observation suggests that the C-terminal 57 amino acid domain is required for the DNA binding property of this protein but not for its PTPase activity.

PTP-1 Does Not Bind to DNA. PTP-1 and PTP-S are nontransmembrane PTPases which show 74% homology in the catalytic domain but very little homology in the carboxy-terminal noncatalytic domain. In order to determine whether PTP-1 also binds to DNA, this protein was expressed in *E. coli* under the control of the *trc* promoter by constructing the vector pKK-PTP-1 as described under Materials and Methods. PTP-1 expressed in bacteria was enzymatically active (data not shown) and showed a protein of 50 kDa on a polyacrylamide gel (Figure 9A). Southwestern blot analysis showed that PTP-1 did not bind to DNA (Figure 9B). Since the level of expression of the PTP-1 protein was less than that of PTP-S, the blots were exposed longer. Even then, no binding of DNA to PTP-1 could be detected. This is not surprising since the stretches of basic amino acids present in the carboxy-terminal domain of PTP-S are not present in PTP-1.

Characterization of the Interaction of PTP-S with DNA. The interaction of the PTP-S gene product expressed in bacteria (and the fusion proteins with GST) with DNA was examined under a variety of conditions of salt, buffer, and pH using the Southwestern blotting method. Binding occurred in Tris as well as phosphate buffers. The binding was dependent on pH and salt concentration. At pH 6, binding of double-stranded and single-stranded DNA was much stronger than at pH 7 or 8 (data not shown). Binding was much more at lower salt concentration (40 mM NaCl) than at higher salt concentration (150 mM NaCl or 40 mM sodium phosphate, pH 7.4). In all these experiments, rat genomic DNA, labeled by nick-translation, was used. At 40 mM NaCl concentration, many *E. coli* proteins also showed binding to double-stranded as well as single-stranded DNA (data not shown). A prominent *E. coli* protein of about 80 kDa showed preferential binding to single-stranded DNA even at 150 mM NaCl concentration (Figures 3 and 7). Plasmid pUC-19, λ DNA, and rat genomic DNA showed nearly similar binding to the PTP-S gene product of 47 kDa and to the fusion proteins with GST. Single-stranded DNA usually showed a slightly higher level of binding as compared to double-stranded DNA (Figures 3 and 7). This could be due to a 2-fold higher molar concentration of single-stranded DNA since an equal amount of DNA by weight was used for binding experiments.

The binding of DNA fragments of different sizes to PTP-S was analyzed using the gel retardation assay. For this purpose, the PTP-S gene product expressed in bacteria and the 41-kDa fusion protein with GST were purified by electroelution from polyacrylamide gels. These electroeluted proteins retained the capacity to bind to DNA in Southwestern blotting experiments (figure not shown). The *Hin*fI digest of pBR322 was incubated with PTP-S or the 41-kDa fusion proteins and analyzed on a polyacrylamide gel (Figure 10). The larger fragments of pBR 322 were bound preferentially by these proteins. This observation suggests that the binding of PTP-S protein to DNA is nonspecific since larger DNA fragments would be expected to have more binding sites than the smaller ones.

DISCUSSION

The results presented here suggest that the PTP-S gene product made in *E. coli* binds to double-stranded DNA at pH 7.1–7.4 and moderate salt concentration. Its affinity for DNA is largely due to nonspecific interactions since it binds to both single-stranded and double-stranded DNA equally well. The affinity of this protein for DNA appears to be fairly high

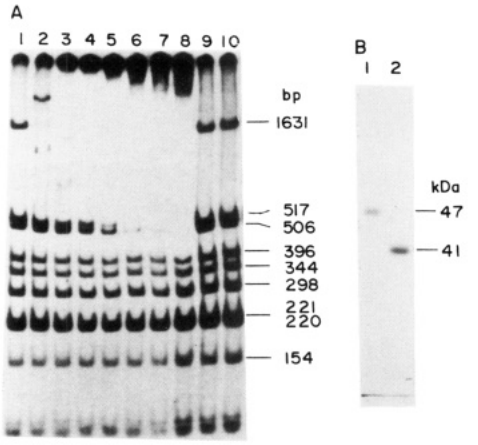

FIGURE 10: Analysis of PTP-S protein binding to DNA by the gel retardation method. Varying amounts of PTP-S protein made in bacteria (purified by electroelution) were incubated with the *Hin*fI digest of pBR322 in 40 mM Tris–acetate, pH 7.4, and 1 mM EDTA and analyzed by electrophoresis in an acrylamide gel as described under Materials and Methods. Lane 1, control, no protein; lane 2, 8 pmol; lane 3, 12 pmol; lane 4, 16 pmol; lane 5, 24 pmol; lane 6, 32 pmol; lane 7, 40 pmol (lanes 2–7, PTP-S protein); lane 8, 80 pmol of 41-kDa fusion protein with GST; lane 9, 300 pmol bovine serum albumin; lane 10, no protein control. (B) Polyacrylamide gel electrophoresis in the presence of SDS of purified PTP-S protein (lane 1) and GST fusion protein of 41 kDa (lane 2).

since 0.4 M NaCl is required for elution from the DNA–Sepharose column. DNA binding studies by Southwestern blotting also suggest that the affinity of PTP-S for DNA is fairly high since, under our experimental conditions, lactate dehydrogenase (Figure 7) and glyceraldehyde-3-phosphate dehydrogenase (data not shown) did not show any binding to DNA even after extended periods of autoradiography. Many eukaryotic sequence-specific DNA binding proteins such as the chick oviduct progesterone receptor, yeast gal-4 transcriptional activator, and human *c-myc* gene product also bind nonspecifically to DNA with high affinity (Hughes et al., 1981; Persson & Leder, 1984; Watt et al. 1985). It has been suggested that high nonspecific affinity for double-stranded DNA is a general property of eukaryotic DNA binding regulatory proteins (Watt et al., 1985).

The cDNA-derived amino acid sequence of the PTP-S gene product suggests that the probable DNA binding domain of this protein is the carboxy-terminal noncatalytic region. The observation that the carboxy-terminal 57 amino acids of this protein confer DNA binding property on GST is consistent with the above suggestion. The significance of the DNA binding property of this phosphatase is not known at present. Inability of PTP-1 to bind to DNA suggests that DNA binding is not a general property of PTPases.

Phosphorylation at Tyr residues is known to occur in some DNA binding proteins such as the receptors for estradiol (Migliaccio et al., 1986) and glucocorticoid (Rao & Fox, 1987). Abelson murine leukemia virus transformed cells have been found to contain elevated levels of several phosphotyrosyl proteins which bind to DNA; these are present at a low level in normal cells (Bell et al., 1987). PTPases which bind to DNA may be required for the dephosphorylation of DNA-bound phosphotyrosyl proteins. To our knowledge, such PTPases have not so far been described. The results of in vitro experiments described in this paper raise the possibility that the phosphatase coded by PTP-S may be such an enzyme. However, this possibility needs to be explored further by more direct or in vivo experiments.

A protein-tyrosine phosphatase, p65, has been identified as a component of human M phase-promoting factor (Meinkrantz et al., 1991). This enzyme, which is a glycoprotein, is localized in the nucleus and is inhibited completely by dithiothreitol. The gene coding for p65 has not been identified, and it is not known whether it binds to DNA or not. The properties of this enzyme such as inhibition by dithiothreitol and substrate specificity suggest that it is not likely to be the product of human T-cell PTPase. The CDC25 gene product, which is required for the entry of the cells into mitosis, is a PTPase localized in the nucleus during interphase (Miller et al., 1991). Whether the CDC25 gene product binds to DNA or not is not known.

The carboxy-terminal 35 amino acids of PTP-1B are required for targeting to endoplasmic reticulum (Frangioni et al., 1992). It seems possible that noncatalytic domains of other nontransmembrane PTPases may be involved in interacting with some cellular component. The carboxy-terminal 11-kDa segment of human T-cell PTPase is required for interacting with some unidentified cellular component (Cool et al., 1990). Although the subcellular location of T-cell PTPase or PTP-S is not known, the presence of a nuclear location signal in the carboxy-terminal region has been pointed out (Cool et al., 1990; Swarup et al., 1991). The results presented here on the in vitro interaction of the PTP-S gene product with DNA raise the possibility that PTP-S may interact with DNA in the cell also.

The DNA binding property of the PTP-S gene product raises the possibility that the homology with basic domains of Fos and Jun may have functional significance. One possibility is that the PTP-S gene product may function as a PTPase on the DNA-bound phosphotyrosylproteins. However, it is worthwhile to mention certain properties which the PTP-S gene product shares with many sequence-specific DNA binding proteins which function as transcription factors. These are the following: (a) organization of the basic region in the cluster–spacer–cluster pattern which is also found in many sequence-specific DNA binding proteins (Busch & Sassone-Corsi, 1990); (b) an increase in the level of mRNA on treatment with cycloheximide (Swarup et al., 1991); (c) the short half-life of mRNA of about 20 min (unpublished observations); (d) nonspecific binding to DNA; (e) the presence of a domain with acidic and serine/threonine-rich stretches, preceding the basic domain (Ser-301 to Ser-323). This domain also contains potential phosphorylation sites for casein kinases I and II (Swarup et al., 1991). Although the exact role of the PTP-S gene product is not known, the identification of its DNA binding property adds a new element to the growing structural and perhaps functional diversity of these enzymes (Guan & Dixon, 1990; Gu et al., 1991; Guan et al., 1991; Shen et al., 1991; Yang & Tonks, 1991).

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