Novel putative protein tyrosine phosphatases identified by the polymerase chain reaction

Masahiro Nishi, Shinya Ohagi and Donald F. Steiner

The Department of Biochemistry and Molecular Biology and The Howard Hughes Medical Institute, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637, USA

Received 8 June 1990

Protein tyrosine phosphatases (PTPases) are a family of enzymes that specifically dephosphorylate phosphotyrosyl residues in selected protein substrates. To more fully understand the regulatory role of protein tyrosine phosphorylation and dephosphorylation in cellular signal transduction, characterization of PTPases is essential. Using the polymerase chain reaction and degenerate oligonucleotide primers corresponding to conserved amino acid sequences within the catalytic domain of PTPases, we have identified 11 PTPase-related human liver cDNA sequences. Five of these have not been described previously. These results indicate that, like protein tyrosine kinases, PTPases may also comprise a gene family with a large number of members.

Protein tyrosine phosphatase (human liver); Gene family; Polymerase chain reaction; Tyrosine phosphorylation; Growth regulatory gene

1. INTRODUCTION

Protein tyrosine phosphorylation and dephosphorylation are important cellular regulatory mechanisms in the response to hormones and growth factors and in the control of cell growth [1,2]. Until relatively recently, much attention has been focused on protein tyrosine phosphorylation and protein tyrosine kinases (PTKases). Growth factor receptors such as the insulin and epidermal growth factor receptors possess an intrinsic PTKase activity that is activated by ligand binding. Moreover, a number of oncogene products also have PTKase activity. In order to study the physiological role of protein tyrosine phosphorylation systems more precisely, both the process of phosphorylation as well as its reversal process, i.e. the removal of phosphates from phosphotyrosyl residues, must be well understood. Protein tyrosine phosphate phosphohydrolases (PTPases: EC 3.1.3.48) are recently discovered cellular enzymes which catalyze this reverse process [3]. It is thus evident that for a more complete description of these cellular regulatory circuits it will be important to characterize not only relevant PTKases but also their corresponding PTPases. Several such PTPases have been characterized in recent studies [4–9]. The placental PTPase 1B is a 37 kDa protein having a 237 residue conserved catalytic core [4]. Analysis of its cDNA, however, predicts a 435 aa protein which has an additional hydrophobic C-terminal segment of approx. 114 aa which may regulate its activity or localization [8]. Other PTPases have been found to have a transmembrane segment and contain 2 tandem repeats of the core catalytic domain (for a review see [3]). PTPases can thus be tentatively classified into two subgroups, transmembrane and cytoplasmic PTPases. To identify new members of this emerging family, we have employed the polymerase chain reaction (PCR) using human liver cDNA and primers based on conserved sequence elements in the known enzymes. We report here the identification of 11 hepatic PTPase-related sequences, including five that are novel.

Correspondence address: D.F. Steiner, Howard Hughes Medical Institute, The University of Chicago, 5841 S. Maryland Avenue, Box 23, Chicago, IL 60637, USA

2. MATERIALS AND METHODS

2.1. *Materials*

Oligonucleotides were synthesized on an Applied Biosystems model 380B DNA synthesizer. RNAs were prepared from human and rat liver by the guanidinium thiocyanate/CsCl procedure [10].

2.2. *Polymerase chain reaction (PCR)*

RNAs were converted to single strand cDNAs by antisense primer (5′-GGCCA$^{A}_{C}$TA$^{T}_{C}$TGI$^{G}_{T}$C$^{A}_{G}$CA$^{T}_{C}$TT-3′) and avian myeloblastosis virus reverse transcriptase. This cDNA was used as a template for in vitro enzymatic amplification with *Thermus aquaticus* (Taq) DNA polymerase and sense (5′-GGITCIGA$^{T}_{C}$TA$^{T}_{C}$ATIAA$^{T}_{C}$GC-3′) and antisense primers. The PCR was carried out in a Perkin-Elmer Cetus DNA thermal cycler through 30 cycles. The 1st and 2nd cycles were for 1 min at 94°C (denaturation), 2 min at 37°C (annealing) and 2 min at 72°C (extension). The following cycles were carried out under the same conditions except for the annealing temperature (3rd and 4th cycles at 45°C and 5th–last cycles at 55°C). The PCR products were analyzed on 4% Nuseive GTG agarose (FMC

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

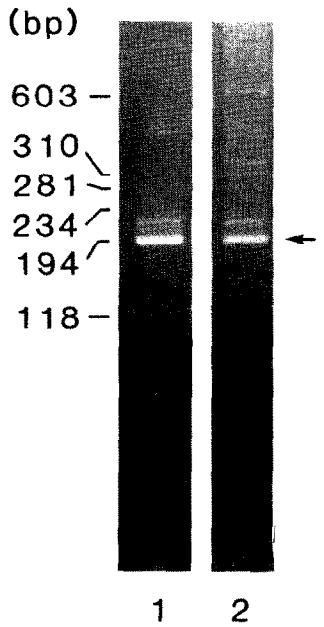

Fig. 1. Analysis by agarose gel electrophoresis of PCR products. Aliquots (10 μl) of PCR products from human (lane 1) and rat liver cDNA (lane 2) were analyzed on 4% Nuseive GTG agarose gel. The size markers are shown at the left. The expected size of 191 bp for the products is indicated by an arrow.

Bioproducts, ME, USA) gel and DNA fragments of the expected size (191 bp) were excised, eluted from the gel, subcloned into the *Hin*cII site of pGEM4Z (Promega, Madison, WI, USA) and sequenced by the dideoxynucleotide chain-termination procedure [11].

3. RESULTS

The PCR primers were designed corresponding to conserved regions of the PTPase catalytic domains [3]. The expected fragment size of 191 bp was not the only band seen but was the major product obtained in the PCR of both human and rat liver cDNA (Fig. 1). Sequence analysis of the 191 bp fragment from human liver cDNA revealed 11 different PTPase-related clones (Figs 2, 3). PTP191-3, -5, -17, -22 and -30 are identical to the leukocyte common antigen (LCA, also called CD45 or T200), domain 1 [12], LAR domain 2 [13], PTPase 1B [4,8], T-cell PTPase [5] and LAR domain 1 [13], respectively. PTP191-2 has an identical predicted amino acid sequence to mouse LRP domain 1 [9], suggesting that it is a human homologue of LRP. The other five clones however, are novel (Table I). Of 45 sequenced PTPase-related clones, PTP191-1 and -2 were the most frequent; nine of each clones were obtained, while only one clone was obtained for PTP191-10 and -33 (Table I). This frequency may

```
PTP191-1  CAGCTACATCCCTGGCAACAACTTCAGAAGAGAATACATTGTCACTCAGGGACCGCTTCCTGGCACCAAGGATGACTTCT
     -2   TTCATTCATCAACGGTTACCAAGAAAAGAACAAATTCATTGCTGCACAAGGACCAAAAGAAGAAACGGTGAATGATTTCT
     -3   CAGCTATATTGATGGTTTCAAAGAACCCAGGAAATACATTGCTGCTCAAGGTCCCAGGGATGAAACTGTTGATGATTTCT
     -5   CAGCTTCCTGGATGGTTATAGACAGCAGAAGGCCTACATAGCTACACAGGGGCCTCTGGCAGAGAGCACCGAGGACTTCT
    -10   CAGTTTTATTGATGGATACAGACAACAGAAAGCCTACATCGCTACCCAGGGGCCCTTGGCAGAGACCACTGAAGACTTCT
    -13   AAACTATGTTGATGGTTACAACAAAGCAAAAGCCTACATTGCCACCCAAGGACCTTTGAAGTCTACATTTGAAGATTTCT
    -17   TAGTTTGATAAAAATGGAAGAAGCCCAAAGGAGTTACATTCTTACCCAGGGCCCTTTGCCTAACACATGCGGTCACTTTT
    -22   CAGTTTAGTTGACATAGAAGAGGCACAAAGGAGTTACATCTTAACACAGGGTCCACTTCCTAACACATGCTGCCATTTCT
    -30   CAACTACATCGATGGCTACCGCAAGCAGAATGCCTACATCGCCACGCAGGGCCCCCTGCCCGAGACCATGGGCGATTTCT
    -33   CAACTATATTGATGGCTACCAGAGACCAAGTCATTACATTGCAACCCAAGGTCCCGTTCATGAAACAGTGTATGATTTCT
    -36   TTCCTACATAGATGGTTACAAAGAGAAGAATAAATTCATAGCAGCTCAAGGTCCCAAACAGGAAACGGTTAACGACTTCT

PTP191-1  GGAAAATGGTGTGGGAACAAAACGTTCACAACATCGTCATGGTGACCCAGTGTGTTGAGAAGGGCCGAGTA
     -2   GGCGGATGATCTGGGAACAAAACACAGCCACCATCGTCATGGTTACCAACCTGAAGGAGAGAAAGGAGTGC
     -3   GGAGGATGATTTGGGAACAGAAAGCCACAGTTATTGTCATGGTCACTCGATGTGAAGAAGGAAACAGGAAC
     -5   GGCGCATGCTATGGGAGCACAATTCCACCATCATCGTCATGCTGACCAAGCTTCGGGAGATGGGCAGGGAG
    -10   GGCGGATGCTCTGGGAACACAATTCCACCATAGTTGTGATGCTCACCAAGCTGCGTGAAATGGGCAGAGAG
    -13   GGAGGATGATTTGGGAACAAAACACTGGAATCATTGTGATGATTACGAACCTTGTGGAAAAAGGAAGACGA
    -17   GGGAGATGGTGTGGGAGCAGAAAAGCAGGGGTGTCGTCATGCTCAACAGAGTGATGGAGAAAGGTTCGTTA
    -22   GGCTTATGGTTTGGCAGCAGAAGACCAAAGCAGTTGTCATGCTGAACCGCATTGTGGAGAAAGAATCGGTT
    -30   GGAGAATGGTGTGGGAACAGCGCACGGCCACTGTGGTCATGATGACACGGCTGGAGGAGAAGTCCCGGGTA
    -33   GGAGGATGATTTGGCAAGAACAATCTGCTTGCATTGTGATGGTTACAAATTTAGTTGAGGTTGGCCGGGTT
    -36   GGAGAATGGTCTGGGAGCAAAAGTCTGCGACCATCGTCATGTTAACAAACTTGAAAGAAAGGAAAGAGGAA
```

Fig. 2. Nucleotide sequences of human liver PTPase-related partial cDNA clones. The PCR primer sequences are not shown.

```
                                                                          Reference
PTP1B (Human)   DNDYINASLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVVMLNRVMEKGSLKCAQYWP  [4,8]
PTP-1 (Rat)     DNDYINASLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVVMLNRIMEKGSLKCAQYWP  [7]
TCPTP (Human)   ENDYINASLVDIEEAQRSYILTQGPLPNTCCHFWLMVWQQKTKAVVMLNRIVEKESVKCAQYWP  [5]
LCA-D1 (Human)  GSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMVTRCEEGNRNKCAEYWP  [12]
LAR-D1 (Human)  GSDYINANYIDGYRKQNAYIATQGPLPETMGDFWRMVWEQRTATVVMMTRLEEKSRVKCDQYWP  [13]
DLAR-D1 (Dm)    GSDYINANYCDGYRKHNAYVATQGPLQETFVDFWRMCWELKTATIVMMTRLEERTRIKCDQYWP  [6]
DPTP-D1 (Dm)    TTDYINANFVIGYKERKKFICAQGPMESTIDDFWRMIWEQHLEIIVILTNLEEYNKAKCAKYWP  [6]
LRP-D1 (Mouse)  DSQYINASFINGYQEKNKFIAAQGPKEETVNDFWRMIWEQNTATIVMVTNLKERKECKCAQYWP  [9]
LCA-D2 (Mouse)  PSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKHGDQEICAQYWG  [12]
LAR-D2 (Mouse)  GSDYINASFLDGYRQQKAYIATQGPLAESTEDFWRMLWEHNSTIIVMLTKLREMGREKCHQYWP  [13]
DLAR-D2 (Dm)    GSDYVNASFIDGYRYRSAYIAAQGPVQDAAEDFWRMLWEHNSTIVVMLTKLKEMGREKCFQYWP  [6]
DPTP-D2 (Dm)    NSTYINASFIEGYDNSETFIIAQDPFENTIGDFWRMISEQSVTTLVMISEIGD:GPRKCPRYWA  [6]
LRP-D2 (Mouse)  NTDYVNASFIDGYRQKDSYIRSQGPLLMTIEVFWRMIWEWKSCSIVMLTELEERGQEKCAQYWP  [9]

PTP191-1              SYIPGNNFRREYIVTQGPLPGTKDDFWKMVWEQNVHNIVMVTQCVEKGRV
   191-2              SFINGYQEKNKFIAAQGPKEETVNDFWRMIWEQNTATIVMVTNLKERKEC
   191-3              SYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMVTRCEEGNRN
   191-5              SFLDGYRQQKAYIATQGPLAESTEDFWRMLWEHNSTIIVMLTKLREMGRE
   191-10             SFIDGYRQQKAYIATQGPLAETTEDFWRMLWEHNSTIVVMLTKLREMGRE
   191-13             NYVDGYNKAKAYIATQGPLKSTFEDFWRMIWEQNTGIIVMITNLVEKGRR
   191-17             SLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVVMLNRVMEKGSL
   191-22             SLVDIEEAQRSYILTQGPLPNTCCHFWLMVWQQKTKAVVMLNRIVEKESV
   191-30             NYIDGYRKQNAYIATQGPLPETMGDFWRMVWEQRTATVVMMTRLEEKSRV
   191-33             NYIDGYORPSHYIATQGPVHETVYDFWRMIWQEQSACIVMVTNLVEVGRV
   191-36             SYIDGYKEKNKFIAAQGPKQETVNDFWRMVWEQKSATIVMLTNLKERKEE
```

Fig. 3. Comparison of predicted amino acid sequences of known PTPases and human liver PTPase-related partial cDNAs.

Table I

Type and frequency of human liver PTPase-related clones

Clone	Type	Number[a]
PTP191-1	novel	9
PTP191-2	LRP-D1	9
PTP191-3	LCA-D1	5
PTP191-5	LAR-D2	8
PTP191-10	novel	1
PTP191-13	novel	3
PTP191-17	PTPase 1B	2
PTP191-22	T-cell PTPase	2
PTP191-30	LAR-D1	3
PTP191-33	novel	1
PTP191-36	novel	2

[a] A total of 45 clones were examined

represent the mRNA abundance of each clone in human liver.

4. DISCUSSION

Protein tyrosine phosphorylation and dephosphorylation have been implicated as important cellular regulatory mechanisms and are catalyzed by PTKases and PTPases respectively. While there has been considerable progress in characterizing PTKases, until recently, relatively little has been known concerning tyrosine dephosphorylating enzymes. Since the isolation and the determination of the amino acid sequence of placental PTPase 1B [3,4], however, cDNA cloning of several PTPases has been reported [5–9] and evidence that PTPases like PTKases also constitute a large gene family has been accumulating. For the identification of novel members of a gene family PCR has strong advantages and successful results in some other gene families have already been reported [14,15]. We report here partial sequences of 11 different PTPase-related proteins, including five novel ones identified by means of PCR using liver cDNA.

The use of oligonucleotide primers corresponding to different conserved motifs or the use of different RNA sources may yield additional members of the PTPase gene family. The particular functions of individual PTPases are still unknown. Several membrane-anchored PTPases are similar to cell adhesion molecules. Their extracellular domains have immunoglobulin-like repeats and fibronectin type III-like repeats. Based on these features, they are presumed to function via cell-cell or cell-matrix interactions [6]. Another transmembrane PTPase, LCA may play a role in T-cell signal transduction by dephosphorylating T-cell PTKase $pp56^{lck}$ [16,17]. PTPase 1B, a cytoplasmic type PTPase, may dephosphorylate the β subunit of the insulin receptor. The injection of this enzyme into *Xenopus* oocytes antagonized insulin action and reduced the phosphorylation of insulin receptor β subunits [3,18]. For the functional characterization of new members of the PTPase family, the isolation and expression of full length cDNA clones will be necessary. These results provide a basis for such further studies.

REFERENCES

[1] Hunter, T. and Cooper, J.A. (1985) Annu. Rev. Biochem. 54, 897–930.
[2] Yarden, Y. and Ullrich, A. (1988) Annu. Rev. Biochem. 57, 443–478.
[3] Tonks, N.K. and Charbonneau, H. (1989) TIBS 14, 497–500.
[4] Charbonneau, H., Tonks, N.K., Kumar, S., Diltz, C.D., Harrylock, M., Cool, D.E., Krebs, E.G., Fischer, E.H. and Walsh, K.A. (1989) Proc. Natl. Acad. Sci. USA 86, 5252–5256.
[5] Cool, D.E., Tonks, N.K., Charbonneau, H., Walsh, K.A., Fischer, E.H. and Krebs, E.D. (1989) Proc. Natl. Acad. Sci. USA 86, 5257–5261.
[6] Streuli, M., Krueger, N.X., Tsai, A.Y.M. and Saito, H. (1989) Proc. Natl. Acad. Sci. USA 86, 8698–8702.
[7] Guan, K., Haun, R.S., Watson, S.J., Geahlen, R.L. and Dixon, J.E. (1990) Proc. Natl. Acad. Sci. USA 87, 1501–1505.
[8] Chernoff, J., Schievella, A.R., Jost, C.A., Erikson, R.L. and Neel, B.G. (1990) Proc. Natl. Acad. Sci. USA 87, 2735–2739.
[9] Matthews, R.J., Cahir, E.D. and Thomas, M.L. (1990) Proc. Natl. Acad. Sci. USA 87, 4444–4448.
[10] Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W.J. and Goodman, H.M. (1977) Science 239, 487–491.
[11] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[12] Ralph, S.J., Thomas, M.L., Morton, C.C. and Trowbridge, I.S. (1987) EMBO J. 6, 1251–1257.
[13] Streuli, M., Krueger, N.X., Hall, L.R., Schlossman, S.F. and Saito, H. (1988) J. Exp. Med. 168, 1523–1530.
[14] Wilks, A.F. (1989) Proc. Natl. Acad. Sci. USA 86, 1603–1607.
[15] Betsholtz, C., Baumann, A., Kenna, S., Ashcroft, S.J.H., Berggren, P.-O., Grupe, A., Pongs, O., Rorsman, P., Sandblom, J. and Welsh, M. (1990) FEBS Lett. 263, 121–126.
[16] Mustelin, T., Coggeshall, K.M. and Altman, A. (1989) Proc. Natl. Acad. Sci. USA 86, 6302–6306.
[17] Ostergaard, H.L., Shackelford, D.A., Hurley, T.R., Johnson, P., Hyman, R., Sefton, B.M. and Trowbridge, I.S. (1989) Proc. Natl. Acad. Sci. USA 86, 8959–8963.
[18] Cicirelli, M.F., Tonks, N.K., Diltz, C.D., Weiel, J.E., Fischer, E.H. and Krebs, E.G. (1990) Proc. Natl. Acad. Sci. USA 87, 5514–5518.