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Human placenta protein-tyrosine-phosphatase: Amino acid sequence and relationship to a family of receptor-like proteins

(sequence homology/catalytic domains/CD45)

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ABSTRACT **The amino acid sequence of the cytosolic human placenta protein-tyrosine-phosphatase 1B (PTPase 1B; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) has been determined. It consists of a single chain of 321 residues with an N-acetylated N-terminal methionine and an unusually proline-rich C-terminal region. The enzyme is structurally related to the two cytoplasmic domains of both the leukocyte common antigen CD45 and LAR, a CD45-like molecule with an external segment that resembles a neural cell adhesion molecule. A low molecular weight protein encoded by a cDNA clone from T cells also shows extensive sequence similarities. The present study defines homologous domains common to this diverse family of PTPases that includes both soluble and receptor-like transmembrane forms. The cysteinyl residues 121 and 215 of PTPase 1B are conserved among all members of the family and are candidates for involvement in catalysis since PTPase 1B is inactivated by thiol modifying reagents. Two segments rich in positively charged residues (residues 33–47 and 227–238) may provide sites of interaction with inhibitory anionic polymers such as heparin or poly(Glu/Tyr).**

Changes in the state of phosphorylation of tyrosyl residues in proteins have been implicated in a number of cellular processes, including cell growth, differentiation, and oncogenic transformation (1). However, the identity of the protein substrates and the mechanism by which their phosphorylation and dephosphorylation mediates phenotypic responses remain to be fully defined.

While there has been considerable progress in characterizing the kinases, only recently have the protein-tyrosine-phosphatases (PTPases; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) been purified to homogeneity (2). Of the several PTPase isozymes observed in human placenta extracts, one, PTPase 1B, has been isolated as a M_r 37,000 monomeric catalytically active protein. It acts exclusively on phosphotyrosyl residues, displaying a high affinity and specific activity toward an artificial substrate derived from lysozyme.

In the course of the present study, it was noted that a segment of the sequence of PTPase 1B bore a striking similarity to the intracellular domains of a large transmembrane protein, the leukocyte common antigen CD45 (3). CD45 represents a broad family of proteins of M_r 180,000–240,000 found on the surface of all hematopoietic cells except mature erythrocytes and differing in their extracellular N-terminal structure. It has been proposed that these proteins modulate B- and T-lymphocyte cell responses and alter interleukin 2 receptor expression (4, 5). The CD45 from human spleen has since been shown to have PTPase activity (6). Recently, a related molecule, LAR, in various tissues was shown to resemble CD45 and PTPase 1B in its cytoplasmic domains but to have an external segment displaying structural characteristics of a neural cell adhesion molecule (N-CAM) (7). The transmembrane proteins CD45 and LAR have extracellular segments with characteristics of ligand-binding structures indicating that they may be receptor-linked PTPases (3, 6, 8).

In the accompanying manuscript (9), Cool *et al.* describe a cDNA from a human T-cell library encoding a M_r 48,000 protein§ that closely resembles PTPase 1B. The present study reports the complete amino acid sequence of PTPase 1B and compares sequences of this family of proteins, drawing attention to the conserved features of a core structure presumed to represent the catalytic domains.

MATERIALS AND METHODS

PTPase 1B was isolated as described (2), lyophilized or precipitated with trichloroacetic acid, and then reduced and pyridylethylated (10) or carboxymethylated (11) in preparation for fragmentation.

Trypsin was obtained as TPCK-trypsin from Cooper Biomedical and *Staphylococcus aureus* protease from Miles; *Achromobacter* protease I was a generous gift of T. Masaki (Ibaraki University, Japan). The narrow bore columns (Butyl-300, RP-300, RP-8, and RP-18) were obtained from Pierce.

Aliquots (5 nmol) of *S*-carboxymethyl protein were digested with *Achromobacter* protease I for 14 hr at 37°C in 3.1 M urea/50 mM Tris·HCl, pH 9.0, using an enzyme/substrate ratio of 1:300 (wt/wt). *S*-pyridylethyl protein (4 nmol) was citraconylated (12), dialyzed against 50 mM NH_4HCO_3 (pH 8.8) and cleaved at arginyl residues with 1% (wt/wt) trypsin for 3 hr at 37°C. Digestion was terminated by adding formic acid.

Acid cleavage at aspartyl residues was carried out in 2% formic acid for 4.5 hr at 110°C under vacuum (13). Cleavage at methionyl residues followed the procedures of Gross (14).

Peptides were purified by size exclusion on two tandem TSK-3000 PW columns (LKB) using 40% acetonitrile in 0.1% trifluoroacetic acid. When necessary, pooled mixtures of peptides were further purified by reverse-phase HPLC on RP-18 (2.1 × 30 mm) or RP-300 (2.1 × 100 mm) columns.

Amino acid analyses followed the phenylisothiocyanate procedure (15), but compositions were used only as a tentative guide to the location and purity of peptides. All of the proof of structure except at the amino terminus was derived

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Abbreviations: PTPase, protein-tyrosine-phosphatase; N-CAM, neural cell adhesion molecule.

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§The protein encoded by this cDNA is designated here as the T-cell PTPase; however, the protein itself has not been isolated and directly demonstrated to possess intrinsic PTPase activity.

by Edman degradation performed in an Applied Biosystems model 470 sequencer with on-line identification of phenylthiohydantoins (16).

Data bases of sequence information were searched for proteins of similar amino acid sequence. The protein data base release 17.0 of June 30, 1988 (National Biomedical Research Foundation) was examined with the SEARCH program (17). The GenBank DNA data base 56.0 of November 1988 was searched with programs from GENEPRO version 4.2 (Riverside Scientific, Seattle) after translation into each of the six theoretical reading frames.

Protein sequences, or segments thereof, that displayed similarities were aligned optimally using the ALIGN program (17), the mutation data matrix, and a gap penalty of 10. Alignment scores are expressed in units of standard deviation from the mean of 100 randomly generated sequences of the same composition.

RESULTS

Since the amino terminus of PTPase 1B is blocked, Edman degradation could not be carried out on the intact protein. Previous work (11) had shown that peptides generated by cleavage at lysyl residues could be easily separated by HPLC. Thus, such a digest of 5 nmol of *S*-carboxymethyl PTPase 1B was prepared and fractionated on RP-300 columns. Edman degradation of 18 derived peptides placed 245 residues in unique nonoverlapping sequences (Fig. 1) that accounted for 76% of the structure of the protein.

Difficulty was encountered in obtaining peptide K16 (residues 198–237) free of K3, but this was circumvented by isolating K16 from a separate subdigest of a cyanogen bromide fragment, M8 (see below). Peptide K20 was observed only as a minor contaminant of K11. Analysis of the fraction containing peptide K17a revealed the sequence illustrated in Fig. 1 as the major component with an uncleaved Lys-Asp bond (residues 239–240). A minor contaminant lacked the N-terminal Arg-Lys, indicating ≈10% cleavage of that bond by the *Achromobacter* protease.

Of the isolated peptides, only one (K24) lacked lysine, suggesting that it must have been derived from the C terminus. Its amino acid composition indicated seven residues, and these were placed in the sequence Arg-Ile-Leu-Glu-Pro-His-Asn by Edman degradation. A blocked pentapeptide (K1) containing two methionyl residues was also of particular interest. Through the courtesy of Patrick Griffin, Jeffrey Shabanowitz, and Donald Hunt, its sequence was determined with a triple quadrupole mass spectrometer as described by Hunt *et al.* (18). Its structure, N^{α}-acetyl-Met-Glu-Met-Glu-Lys identified it as the amino terminus of the protein.

Upon completion of the sequence analysis, it was apparent that five small peptides, each of four residues or less, had not been recovered from the lysine digest by the HPLC purification procedures applied. These segments were accounted for in overlapping peptides from subsequent digests at methionine or arginine.

Peptides derived from cleavage of *S*-pyridylethyl PTPase 1B at methionine were separated by size, and pooled fractions were further purified by reverse-phase HPLC. All of the expected fragments were recovered except those corresponding to *N*-acetyl-Met and Glu-Met (residues 1–3). The

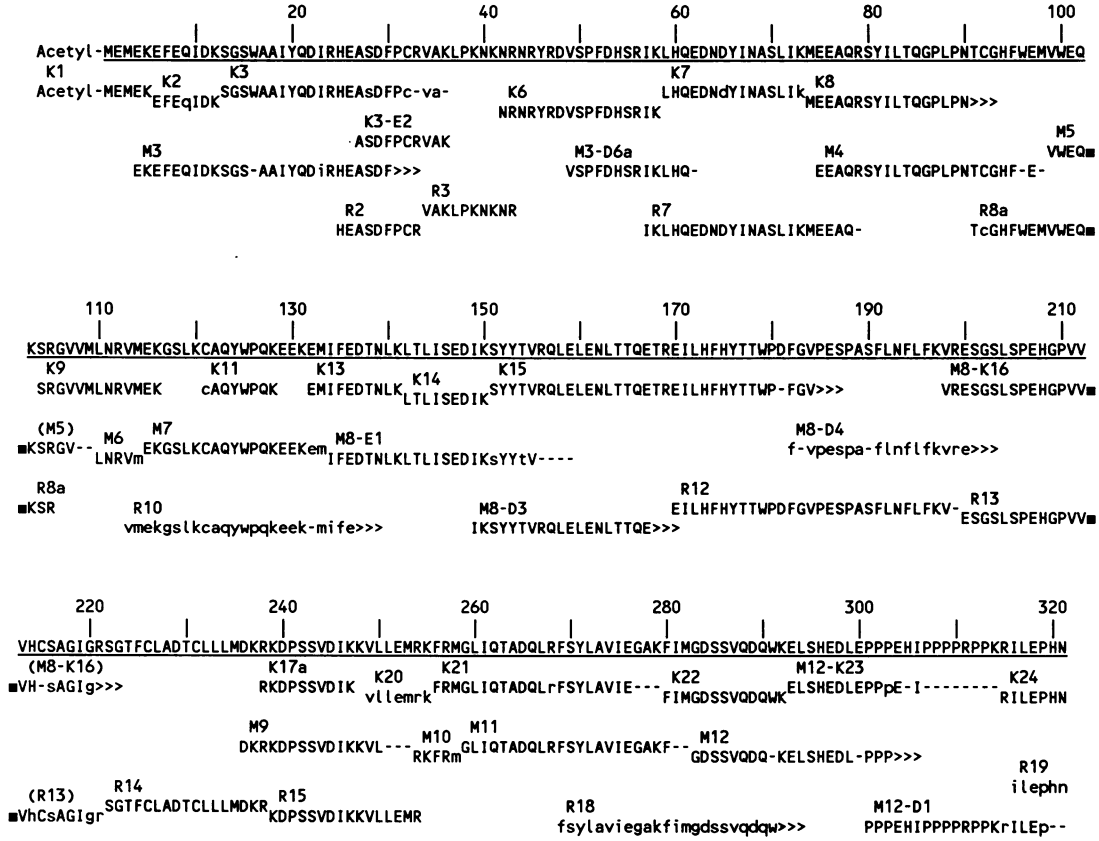

FIG. 1. Detailed summary of the proof of sequence (single-letter code) of human PTPase 1B. The proven sequences of specific peptides are given below the summary sequence (underlined). Prefixes K, R, and M indicate cleavage at Lys, Arg, and Met, respectively. Subpeptides are designated by hyphenated suffixes, where D and E denote cleavage at aspartic acid (with acid) or at glutamic acid (with *S. aureus* protease). Lowercase letters in sequences indicate that the identification of the residue by Edman degradation was only tentative. A dash designates an unidentified residue within a peptide. Arrowheads at the end of a sequence indicate that a peptide has not been analyzed to its C terminus. Residue numbers are above the summary sequence. The symbol ■ at the end of a line indicates that the peptide sequence continues from (or to) the adjacent line.

two largest fragments, M3 and M8, were further purified on a Butyl-300 column and then subdigested by cleavage at aspartic acid. M8 was also subdigested with *S. aureus* protease and both M8 and M12 were subdigested with the *Achromobacter* protease. After isolation, the various subpeptides and the smaller primary fragments were each analyzed by Edman degradation and the data are summarized in Fig. 1. The only primary CNBr fragment lacking homoserine was M12, which was assumed to be C terminal. It was unusually rich in proline and Edman degradation was definitive for only 21 cycles. Of several subdigestions tried, the most useful was that involving anomalous cleavage with acid at a Glu-Pro bond (residues 300–301) producing M12-D1 (Fig. 1). Its sequence provided an overlap with K24, verifying its C-terminal location. Overlaps of these various peptides with the lysyl cleavage fragments provided many alignments but a third digest was needed to complete the analysis.

The remaining overlaps were derived from peptides generated by tryptic cleavage of *N*-citraconyl, *S*-pyridylethyl PTPase 1B at arginine residues. The digest was fractionated as for the CNBr peptides and the sequences of 11 of the 19 products are illustrated in Fig. 1. The only peptide lacking arginine (R19) had a composition consistent with the C-terminal sequence determined in K24. Two peptides, R10 and R18, were not completely separated, even on the Butyl-300 column. The sequence of R10 was determined against a contaminating background of 20% R18; R18 was contaminated by 30% R10.

An unexpected cleavage by trypsin at Asn-Thr (residues 90–91) deserves mention. Anomalous cleavages are occasionally observed with trypsin and are thought to reflect secondary specificity characteristics. In this case, the product R8a provided a useful overlap of M4 and M5. Other peptides provided all but one overlap, leaving three problem regions. At Val-198, a single residue overlap aligns R12 and M8-K16. However, analysis of a trace peptide (2 pmol of M8-D4) confirmed the overlap by extending it by two residues. At Arg-254, a single residue overlap of R15 and M10 is supported by the tentative identification of the sequence of K20, which was a minor contaminant of K11. Finally, no overlap was obtained to align R13 to R14 at residues 221–222. However, all other arginine fragments were placed and the sequence provided by this tentative alignment is identical for 12 residues (Fig. 2) with a corresponding segment of the T-cell PTPase (9).

Altogether, the sequence in Fig. 1 accounts for 321 amino acid residues plus an acetyl group for an M_r of 37,354. It contains 13 alanine, 19 arginine, 11 asparagine, 18 aspartic acid, 6 cysteine, 14 glutamine, 30 glutamic acid, 14 glycine, 11 histidine, 18 isoleucine, 29 leucine, 23 lysine, 11 methionine, 14 phenylalanine, 22 proline, 24 serine, 13 threonine, 6 tryptophan, 9 tyrosine, and 16 valine residues. Other than N-acetylation of the amino-terminal methionine, there is no evidence of posttranslational modification, even at Asn-68 and -162, which are commonly glycosylated in extracellular proteins containing such NxS/T sequences.

An exhaustive search of both protein and DNA data bases drew attention to the CD45 family of transmembrane proteins, where two cytoplasmic domains in each bear a striking resemblance to PTPase 1B. We have already reported the

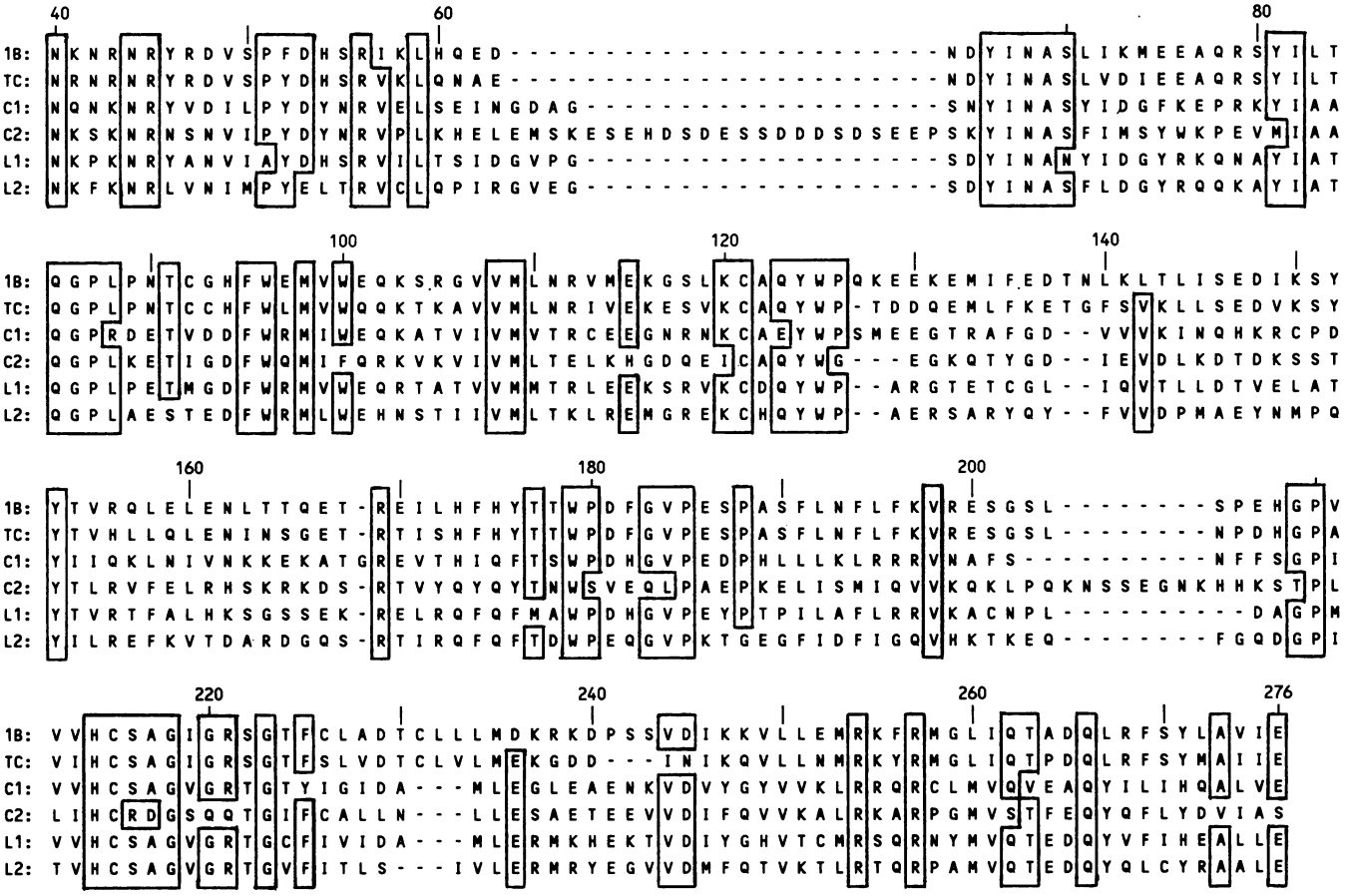

FIG. 2. Alignment of conserved domains from proteins of the PTPase family. The following sequences (single-letter code) are shown: 1B, human placental PTPase 1B; TC, T-cell PTPase (9); C1 and C2, domains I and II of CD45 (19); L1 and L2, domains I and II of LAR (7). Residue numbers are those of PTPase 1B. Boxes identify residues that are identical in at least five of the six chains. Alignment scores (17) vs. PTPase 1B (residues 40–276) are 51.1 for TC (residues 42–274), 24.9 for C1 (residues 491–725), 16.7 for C2 (residues 782–1041), 25.0 for L1 (residues 1349–1580), and 23.1 for L2 (residues 1638–1871) (7, 9, 19). Hyphens denote gaps that optimize the alignment.

Table 1. Alignment scores comparing segments of PTPase 1B with the corresponding segments of its homologs

	PTPase 1B residues					
	1–53	54–106	107–159	160–212	213–265	266–321
T-cell PTPase	14.0	17.8	14.4	20.4	15.6	12.7
CD45 I	4.8	9.5	6.3	8.1	5.1	(0.8)
CD45 II	(1.6)	6.4	3.4	6.1	5.5	(2.0)
LAR I	(2.1)	11.5	6.8	10.0	5.9	(1.9)
LAR II	(2.5)	8.8	6.3	8.3	5.6	(1.5)

Alignment scores express the similarity of segments in units of standard deviation from the averaged background scores of 100 randomly generated sequences (17). Each 53-residue segment of PTPase 1B is compared with a 73-residue segment (including 10 extra residues at each end) from the corresponding region of each of the other proteins. While this tends to reduce the alignment score, it increases the objectivity of the analysis. Scores of <3 (in parentheses) indicate a lack of homology of two segments. Scores of >5 indicate that similarity of sequences suggests homology. Domains of human T-cell PTPase, CD45, and LAR were delineated as follows: T-cell PTPase, residues 1–331 (9); CD45 I, residues 450–800; CD45 II, residues 765–1112 (19); LAR I, residues 1300–1631; LAR II, residues 1603–1881 (7).

similarity of a 157-residue segment of PTPase 1B with corresponding segments of rat and human CD45 later shown to have PTPase activity (3, 6). Table 1 presents the extent of similarity among these molecules including LAR, the recently reported CD45 homolog (7), and a T-cell PTPase described in the companion manuscript (9). In these comparisons, the sequence of PTPase 1B was arbitrarily divided into six ≈53-residue segments. Alignment scores (17) for each paired segment comparison (as defined in Table 1) reveal much greater similarity in the central four segments than at either end except with the T-cell protein. More detailed comparison near the boundaries of the first and last segments indicated that the domain conserved in all four proteins extends from about residues 40 to 276 of PTPase 1B (Fig. 2). Within the family, PTPase 1B and the T-cell PTPase are most similar (Table 1), with 65% sequence identity; that similarity extends from the N terminus virtually to the C terminus (9). The C-terminal segment of PTPase 1B (residues 277–321) is not similar to corresponding segments from the other proteins (Table 1). However, a portion of the N-terminal segment (residues 16–39) of PTPase 1B bears a weak similarity to the corresponding domains I of CD45 and LAR.

DISCUSSION

Approximately 50% of the PTPase activity resists solubilization in the absence of Triton X-100, suggesting that half of the activity is either associated with the membrane or enclosed in vesicles within the cell (11). Very similar peptide fingerprints were seen after digestion of soluble and "particulate"-derived PTPase 1B (11). The present analysis used protein derived separately from each fraction as well as from a 50/50 mixture of both, and all peptides were easily placed within the structure in Fig. 1 without requiring two distinct protein sequences. However, it should be noted that acid-labile posttranslational modifications could have been missed by our techniques that involved acidic steps during fragmentation, purification, and Edman degradation.

The C-terminal 21 residues of PTPase 1B are unusual, containing 48% proline and 33% charged residues. Because this segment is unique, it cannot be essential for catalysis, and it remains to be determined whether it contributes to regulation or localization of the enzyme. PTPase 1B is inhibited by heparin, and two regions are candidates for a complementary polycationic site, residues 33–47 (containing 47% lysine or arginine uninterrupted by acidic side chains) and residues 237–258 (with 36% bearing positive charges). Poly(Glu/Tyr) acts as a noncompetitive inhibitor (11), suggesting that the inhibitor binding site may not coincide with the active site.

It has already been reported that PTPase 1B does not resemble the catalytic subunits of type 1 or 2A Ser/Thr-protein-phosphatases (3), nor does it resemble sequences encoded by a cDNA corresponding to part of the catalytic subunit of murine calcineurin (type 2B), another member of that family (20). The rat type 2C phosphatase sequence (21) is not related to the catalytic subunits of the types 1, 2A, or 2B protein phosphatase or to the PTPase 1B sequence reported here. Thus, there appear to be at least three distinct families of protein phosphatases.

Charbonneau *et al.* (3) reported homology of the intracellular domains of the human and rat CD45 family with a 157-residue segment of PTPase 1B. Streuli *et al.* (7) showed that the intracellular domains of human LAR are homologous with those of CD45. More recently, Cool *et al.* (9) isolated a cDNA that encodes a human T-cell protein having 65% sequence identity with PTPase 1B. Together these make a total of six segments of homologous sequence among four human proteins (Fig. 3). In two of these, PTPase 1B and human CD45, enzymatic activity has been demonstrated (2, 6, 11). Although it has not been shown that LAR or the protein encoded by the T-cell cDNA also expresses PTPase activity, the prediction is as strongly indicated as for CD45. Our present comparison (Fig. 2) of the six complete human sequences demonstrates similarity among all of these structures from Asn-40 to Glu-276 of PTPase 1B.

If one assumes that the minimal segment of demonstrable similarity (residues 40–276 in PTPase 1B) possesses the capacity for independent folding and phosphatase activity,

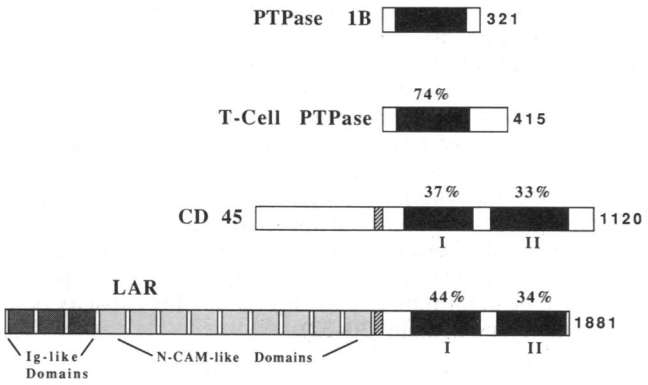

FIG. 3. Diagrammatic alignment of PTPase 1B and related molecules. Solid segments designate conserved domains (from Fig. 2); each is compared with PTPase 1B and the percentage identity is noted. The extracellular segment of LAR is homologous to both immunoglobulin-like and non-immunoglobulin-like domains of N-CAM.

one can identify conserved residues that may be involved in the catalytic and regulatory functions of the enzyme. Several chemically reactive residues are conserved in all six proteins, as shown in Fig. 2. These include Cys-215, Cys-121, His-214, Tyr-66, and Tyr-153; five conserved arginine residues could also be considered. It should be noted that all these residues are also conserved in rat and mouse CD45, for a total of 10 different domains (22, 23). Several of these residues are located within longer conserved segments—e.g., Tyr-66 in Tyr-Ile-Asn-Ala, and His-Cys (residues 214–215) in a segment that is conserved in all but CD45 domain II. This segment, His-Cys-Ser-Ala-Gly-(Ile/Val)-Gly-Arg-(Ser/Thr)-Gly-Xaa-(Phe/Tyr), has a pattern of glycine residues¶ that suggests a break in the secondary structure, possibly a β turn.

Of the conserved residues, Cys-121 and -215 are of particular interest since it has been shown that PTPase 1B is inhibited by reagents that modify thiols (11). The involvement of one or both of these residues in catalysis can be tested by active-site labeling or site-directed mutagenesis.

The alignment of domains in Fig. 2 demonstrates several features that make domain II of CD45 the exception rather than the rule. It possesses unique inserts, 1 of 19 and 1 of 8 residues not present in any of the homologs. The largest of these has characteristics of a phosphorylation site(s) for casein kinase II; as of now, however, no effect of casein kinase II on activity has been detected. The significance of the second insert is unknown. CD45 domain II shows the least similarity to PTPase 1B and the other domains (Table 1 and Fig. 2). In several segments, all domains except CD45 domain II have identical residues. These observations suggest the possibility that this domain may display unique properties.

The structural relationship between PTPase 1B and LAR suggests that the latter may be a receptor-linked PTPase that can function in mechanisms of signal transduction as has been suggested for CD45 (3, 6). The resemblance of the extracellular segment of LAR to that of N-CAM leads to the hypothesis that the ligand in this case may be another LAR molecule on a neighboring cell. If this were the case, and if contact of N-CAM domains on neighboring cells stimulated intracellular PTPase activity, this mechanism could account for contact inhibition of confluent cells, as suggested by Tonks *et al.* (8). Although the Id isotype of N-CAM has a large intracellular domain (27, 28), it bears no resemblance to PTPase 1B. Thus, it is unlikely that the N-CAMs would function in a manner analogous to the hypothetical growth-limiting role of LAR.

The chimeric nature of CD45 and of LAR is evident from the illustration in Fig. 3. The intracellular C-terminal domains are similar and resemble PTPase 1B, but the extracellular N-terminal segments are quite different from each other, implying that primordial gene fusion events may have brought together disparate binding and catalytic capabilities. In recent years, it has become apparent that many other complex proteins have analogous chimeric character whereby the physical linkage of functional units is thought to have introduced synergistic, regulatory, or network-forming characteristics. This has been apparent in multienzyme conjugates (29) and is now evident in certain families such as the protein kinases, where the nature of the regulated response appears to be determined by the ligand-binding capacity of a fused regulatory domain (30–32).

¶Note that the "consensus sequence" Gly-Xaa-Gly-Xaa-Xaa-Gly in enzymes such as the protein kinases has been interpreted as a nucleotide binding site (24, 25); however, this has been controversial (26) and there is no evidence in the present case of an effect of Mg-ATP or GTP on PTPase 1B (11).

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