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Identification of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity associated with the mouse plasma cell differentiation antigen PC-1

(membrane glycoprotein/B lymphocyte/expression vectors)

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ABSTRACT **The protein responsible for both nucleotide pyrophosphatase (EC 3.6.1.9) and alkaline phosphodiesterase I (EC 3.1.4.1) activities was purified from MOPC 315 plasmacytoma cells. A single SDS/PAGE-purified 115-kDa protein band was used to produce a rabbit polyclonal antiserum. This antibody preparation precipitated alkaline phosphodiesterase I activity, indicating that the SDS/PAGE-purified protein was nucleotide pyrophosphatase/alkaline phosphodiesterase I. When used for Western blot analysis, the antiserum detected a 115-kDa protein as well as a 220-kDa protein band. Multiple overlapping cDNA clones were isolated from a cDNA expression library screened with this anti-nucleotide pyrophosphatase/alkaline phosphodiesterase I antiserum. Sequence analysis indicated that the isolated cDNA clones encoded PC-1, a murine plasma cell differentiation antigen. To confirm the suspected enzymatic identity of PC-1, a recombinant PC-1 fusion protein was expressed in bacteria, purified, and used to produce another rabbit polyclonal antiserum. This antiserum likewise immunoprecipitated alkaline phosphodiesterase I activity and recognized the 115-kDa and 220-kDa proteins in Western blot analyses of cell extracts. Furthermore, expression of nucleotide pyrophosphatase/alkaline phosphodiesterase I corresponded directly with mRNA and protein levels of PC-1 in cells known to express different levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity. Finally, steroid induction of enzymatic activity was mirrored by levels of PC-1 mRNA and protein expression. Together, these data indicate that the plasma cell differentiation antigen PC-1 is a membrane-bound enzyme, nucleotide pyrophosphatase/alkaline phosphodiesterase I.**

The pattern of surface antigen expression has been used to identify populations of lymphocytes at various stages of differentiation. A relatively large number of specific B-lineage differentiation antigens characterize B lymphocytes during their development into immunoglobulin-secreting plasma cells. Recently, the functional roles of some of these antigens have been determined. In addition to those with receptor or ligand functions, others have been found to be membrane enzymes that may have important regulatory functions during B-cell maturation (1). For example, the human B-cell antigens CD10, CD45, and CD73 are enzymes possessing neutral endopeptidase, protein-tyrosine-phosphatase, and ecto-5′-nucleotidase activities, respectively (2–4). Most of the known B-cell markers are expressed during early or middle stages of B-cell ontogeny. Only a few antigens expressed during the late events of B-cell differentiation have been identified. In murine B cells, the membrane antigen PC-1 has been found to be expressed at this late stage of B-cell differentiation (5). This differentiation antigen is expressed on immunoglobulin-secreting plasma cells as well as some other, nonlymphoid cells (6). It has been suggested that PC-1 is an enzyme, but its function has not been described (7, 8).

Nucleotide pyrophosphatase (dinucleotide nucleotidohydrolase, EC 3.6.1.9)/alkaline phosphodiesterase I (oligonucleate 5′-nucleotidohydrolase, EC 3.1.4.1) is a membrane protein with a reported molecular weight similar to that of PC-1 (9). A single protein is responsible for both pyrophosphatase and phosphodiesterase enzymatic activities (9–11). We have identified a variant murine plasmacytoma cell line that overexpresses nucleotide pyrophosphatase/alkaline phosphodiesterase I and have shown that this enzyme may have a role in the regulation of N-linked glycosylation (12). To further define regulatory functions, we have purified the protein and have isolated cDNA clones coding for nucleotide pyrophosphatase/alkaline phosphodiesterase I. We now present evidence indicating that nucleotide pyrophosphatase/alkaline phosphodiesterase I is the murine plasma cell differentiation antigen PC-1.

MATERIALS AND METHODS

Purification of Nucleotide Pyrophosphatase/Alkaline Phosphodiesterase I. The cell lines used for these studies were derived from the BALB/c plasmacytoma MOPC 315 (12). Wild-type MOPC 315 cells (MOPC 315/J) synthesize and secrete a trinitrophenol-binding IgA(λ_2) paraprotein. The 315/P cell line is a nonsecretory variant previously shown to have high levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity (12). In some experiments, both cells lines were incubated with dexamethasone since, as has been described for other cell lines (13), we have found this corticosteroid increases specific activity of nucleotide pyrophosphatase/alkaline phosphodiesterase I in MOPC 315 cells (14). Because they express such high levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I, 315/P cells were used as a source for the purification of this membrane protein. Purification was assessed using the substrate thymidine 5′-monophosphate *p*-nitrophenyl ester to detect alkaline phosphodiesterase I activity because of the relative simplicity of this assay (15). The 315/P cells were grown as subcutaneous tumors in BALB/c mice. Approximately 50 g (wet weight) of tumors were removed and disrupted in a Dounce homogenizer. Following centrifugation at 2000 × *g* for 10 min to remove nuclei and unlysed cells, total membranes were isolated (12). The membranes were disrupted in a Dounce homogenizer containing 5 mM $MgCl_2$/1 mM phenylmethanesulfonyl fluoride/1% (vol/vol) Triton X-100/50 mM Tris·HCl, pH 9.0, and alkaline phosphodiesterase I activity was partially purified by Affi-Gel Blue (Bio-Rad) and lentil lectin-Sepharose (Sigma) affinity chromatography using elution with buffers containing NaCl and methyl α-D-

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mannopyranoside, respectively. The lentil lectin affinity-purified enzyme was further purified by Mono Q and Mono P (Pharmacia) column chromatography. Enzyme-containing fractions that were eluted with a linear pH gradient from the Mono P column were concentrated by reapplication to a Mono Q column, and portions of fractions eluted from this Mono Q column with a NaCl gradient were analyzed by SDS/PAGE. The 115-kDa protein band, which correlated with the peak of enzyme activity, was purified to homogeneity by preparative SDS/PAGE followed by electroelution using an Elutrap electro-separation chamber (Schleicher & Schuell).

Preparation of Antisera. Polyclonal antisera against nucleotide pyrophosphatase/alkaline phosphodiesterase I and recombinant PC-1 were produced in New Zealand White rabbits. Immunizations and sera collections were performed following standard protocols (16, 17) except for the primary immunization with the purified 115-kDa nucleotide pyrophosphatase/alkaline phosphodiesterase I. Because of limited amounts of this SDS/PAGE-purified protein, primary immunization was done with an intrasplenic injection of 6 μg of gel-purified protein emulsified in complete Freund's adjuvant. Antisera were assessed for their ability to precipitate alkaline phosphodiesterase I activity. Typically, rabbit antiserum was incubated with partially purified enzyme in 150 mM NaCl/0.1% Triton X-100/10 mM Tris·HCl, pH 7.4. Following incubation for 16 hr at 4°C, 100 μl of a 50% suspension of protein A-Sepharose in buffer without Triton X-100 was added and the incubations were continued for 2 hr. The protein A-Sepharose was collected by centrifugation, washed extensively, resuspended in assay buffer (100 mM NaCl/1.6 mM $MgCl_2$/40 mM Tris·HCl, pH 9.0), and analyzed for alkaline phosphodiesterase I activity. Antiserum used for cDNA library screening was precleared by mixing with an *Escherichia coli* lysate.

Electrophoresis and Western Blotting. Analytical SDS/7.5% PAGE under reducing conditions (18) was performed in a Bio-Rad Mini-PROTEAN II cell. Analyses of the purified 115-kDa protein were performed before and after digestion with 0.25 unit of N-Glycanase (Genzyme). For immunoblotting, proteins separated by SDS/PAGE were transferred to Immobilon-P (Millipore) transfer membranes in 25 mM Tris base/192 mM glycine/20% (vol/vol) methanol at 100 V for 1.5 hr. Following transfer, blots were rinsed in Tris-buffered saline (150 mM NaCl/20 mM Tris·HCl, pH 7.5) containing 0.05% Tween 20 and incubated overnight at room temperature in Tris-buffered saline with 1% bovine serum albumin. The blots were then incubated in this buffer with rabbit polyclonal antibodies for 2 hr. After four washes, the blots were incubated with goat anti-rabbit IgG alkaline phosphatase conjugate (Promega) for 1 hr and developed (19).

Synthesis and Screening of MOPC 315/P cDNA Expression Library in λgtZAP. Because the variant 315/P cells express high levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I (12), they were used as a source for mRNA. Also, these cells were incubated in 200 nM dexamethasone for 48 hr prior to extraction, since this corticosteroid further increases enzymatic specific activity (14). Extraction of total RNA (20), isolation of poly(A)$^+$ RNA (21), and synthesis of double-stranded cDNA (22) were done by standard methods. The cDNA that had been blunt-ended with T4 DNA polymerase was ligated to *Sal* I/*Eco*RI adapters (23), phosphorylated with polynucleotide kinase, and ligated to the dephosphorylated *Eco*RI site of λgtZAP (Stratagene). The recombinant DNA was packaged *in vitro* (Gigapack; Stratagene) to create a library of 1.5×10^5 independent clones. A portion of the cDNA library was amplified and screened using the precleared rabbit antiserum directed against the SDS/PAGE-purified 115-kDa nucleotide pyrophosphatase/alkaline phosphodiesterase I band, followed by a goat anti-rabbit-IgG alkaline phosphatase conjugate (19). Screening of 5×10^5 recombinant phage yielded 5 overlapping cDNA clones. The pBluescript (Stratagene) plasmids containing inserts were isolated from λZAP recombinant clones according to the manufacturer's instructions. The 5′ end of the longest clone, E1A1, was nick-translated and used to further probe the library to isolate another clone (1-2-2) with additional 5′ cDNA sequence. Restriction fragments of the isolated cDNA were subcloned into pBluescript and both strands were sequenced by the dideoxy method of Sanger *et al.* (24), using a modified T7 DNA polymerase (Sequenase; United States Biochemical) and adenosine 5′-[α-[^{35}S]thio]-triphosphate. The order of fragments was established by determining nucleotide sequences across all restriction-site boundaries.

RNA Analysis. Total RNA from cell lines was extracted and purified as described (25). Total RNA samples (20 μg per lane) were electrophoresed in formaldehyde/agarose gels, transferred to nitrocellulose filters (26), and probed with the cDNA insert from E1A1 radiolabeled by nick-translation with [α-^{32}P]dCTP. Hybridization and washing conditions were as described (27).

Expression of cDNA Clones. The cDNA clone 3-1 was subcloned into the expression vector pATH11 (a kind gift from M. Koenig, The Children's Hospital, Boston) and transformed into *E. coli* DH5α. The TrpE–PC-1 fusion protein was isolated by SDS/PAGE following induction with indolacrylic acid (28, 29) and was used to immunize a rabbit as outlined above.

RESULTS

Purification and Immunological Characterization of Nucleotide Pyrophosphatase/Alkaline Phosphodiesterase I. Nucleotide pyrophosphatase/alkaline phosphodiesterase I was purified from variant 315/P plasmacytoma cells that are known to express elevated levels of enzymatic activity. SDS/PAGE analyses after each step of the purification process revealed the progressive enrichment of only two protein bands that correlated with elution of enzymatic activity (unpublished data). The more abundant protein observed had an apparent molecular mass of 115 kDa; in addition, variable amounts of a 220-kDa protein always were detected. This pattern is consistent with the reported molecular weight forms of nucleotide pyrophosphatase/alkaline phosphodiesterase I purified from mouse liver (9). SDS/PAGE analysis of enzyme elution from the second Mono Q column, used to concentrate Mono P fractions, showed only three major protein bands (Fig. 1, lane A). Steps to further purify active nucleotide pyrophosphatase/alkaline phosphodiesterase I resulted in limited sample recovery with significant losses of enzymatic activity. Therefore, preparative SDS/PAGE was used to purify the 115-kDa protein to homogeneity (Fig. 1, lane B). Extensive digestion of the purified protein with N-Glycanase resulted in the generation of a single band at ≈103 kDa, confirming that the 115-kDa protein was a glycoprotein with N-linked oligosaccharides and indicating that only a single protein was present in the gel-purified preparation (Fig. 1, lanes D and E).

A rabbit was immunized with the SDS/PAGE-purified 115-kDa protein to obtain polyclonal antibodies. Western blot analyses of partially purified fractions of nucleotide pyrophosphatase/alkaline phosphodiesterase I revealed that the polyclonal antiserum recognized the 115-kDa band as well as the 220-kDa band (Fig. 1, lane C). That the antiserum raised to the 115-kDa band also recognizes the 220-kDa band indicates that they are immunologically related. Although we have not yet determined the exact molecular relationship of these two protein bands, the higher molecular form most likely represents a nucleotide pyrophosphatase/alkaline

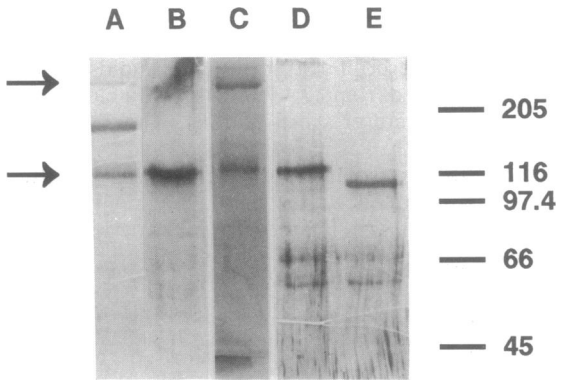

FIG. 1. SDS/PAGE and Western immunoblot analysis of purified nucleotide pyrophosphatase/alkaline phosphodiesterase I. All SDS/PAGE was run under reducing conditions. Lane A, silver stain of Mono Q fraction of peak alkaline phosphodiesterase I activity. Arrows on left indicate positions of major 115- and 220-kDa protein bands corresponding to elution of alkaline phosphodiesterase I activity. Only these two bands correlated with enzymatic activity. Lane B, silver stain of electroeluted, 115-kDa nucleotide pyrophosphatase/alkaline phosphodiesterase I protein band reanalyzed by SDS/PAGE. Lane C, Western immunoblot analysis of lane A fraction. The primary antiserum used was raised to the SDS/PAGE-purified 115-kDa protein indicated in lane B. Lanes D and E, silver stain of SDS/PAGE-purified nucleotide pyrophosphatase/alkaline phosphodiesterase I before and after digestion with N-Glycanase, respectively. Migration of molecular mass markers (kDa) is shown on the right.

phosphodiesterase I dimer as suggested by others (30). If so, dimer formation does not appear to be entirely due to disulfide bonds, since extensive reduction in 2-mercaptoethanol or dithiothreitol failed to alter the SDS/PAGE migration of the purified higher molecular weight form (unpublished data). To confirm that this rabbit polyclonal antiserum recognized active nucleotide pyrophosphatase/alkaline phosphodiesterase I, its ability to immunoprecipitate enzyme activity was analyzed. Table 1 shows that active enzyme can be precipitated by this antiserum, providing additional evidence that the 115-kDa antigen is nucleotide pyrophosphatase/alkaline phosphodiesterase I.

Cloning of Nucleotide Pyrophosphatase/Alkaline Phosphodiesterase I from a cDNA Expression Library. A cDNA expression library was constructed in λgtZAP with mRNA isolated from 315/P plasmacytoma cells, known to express elevated levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity. This library was screened with the rabbitanti-nucleotidepyrophosphatase/alkalinephosphodiesterase I antiserum. Screening of 5×10^5 recombinant phage yielded 5 different, overlapping clones (Fig. 2). Restriction mapping and sequencing of these clones demonstrated that they were derived from a single RNA message and extended

Table 1. Immunoprecipitation of alkaline phosphodiesterase I

Rabbit serum	Activity in immune complexes, nmol/hr
Anti-115-kDa purified protein	
Preimmune	3.3
Immune	76.6
Anti-recombinant PC-1 protein	
Preimmune	1.4
Immune	51.8

Incubation mixtures (10 μl) contained alkaline phosphodiesterase I activity (80 nmol/hr) and antiserum (1 μl). Immune complexes isolated on protein A-Sepharose were collected and assayed directly for alkaline phosphodiesterase I enzymatic activity. Immune antisera inhibited enzymatic activity to varying extents, preventing quantitative recovery of total activity in the protein A-Sepharose pellets.

from the 3′ end of the mRNA (Fig. 2). An *Eco*RI–*Cla* I fragment from the 5′ end of E1A1 was used to rescreen the library to isolate a clone containing more 5′ cDNA sequence. Of several overlapping clones isolated by this second screening, one (1-2-2) extended an additional 461 base pairs toward the 5′ end of the mRNA. The complete nucleotide sequence of the cDNA was determined from clones E1A1 and 1-2-2 and compared with sequences in the GenBank data base (Release 62). Comparison revealed virtual identity with the murine plasmacytoma cell surface antigen PC-1. The most 5′ cDNA clone began at position 122 of this published murine PC-1 sequence (7). However, from more recent evaluation of the human PC-1 sequence, it has been suggested that the major murine transcript may be shorter than originally reported and that the second methionine is the actual initiation methionine (7, 8). Thus, the cDNA described here may contain the complete protein-coding sequence. When compared with the mouse cDNA sequence (7) only three differences, at positions 181 (C to G), 2185 (A to C), and 3193 (C to G), were noted out of 3102 base pairs of nucleotide sequence determined. Only one substitution, at position 2185, changes the coding of an amino acid (methionine to leucine).

Expression of a Nucleotide Pyrophosphatase/Alkaline Phosphodiesterase I (PC-1) Fusion Protein. The initial studies reported above are consistent with the differentiation antigen PC-1 possessing nucleotide pyrophosphatase/alkaline phosphodiesterase I activity. Since both have been reported to have similar molecular weights, the purified 115-kDa antigen could contain both proteins. Therefore, to exclude the possibility that the antiserum raised against the gel-eluted 115-kDa murine protein band contained two populations of antibodies directed against two different proteins, a fragment of PC-1 was expressed in bacteria. The enzymatically inactive TrpE–PC-1 fusion protein was purified by SDS/PAGE and used for immunization of a second rabbit. Thus, any specific antibodies generated could recognize only a single eukaryotic

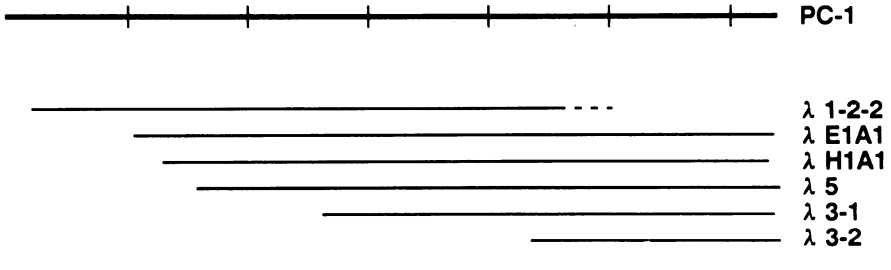

FIG. 2. Schematic representation of PC-1 (nucleotide pyrophosphatase/alkaline phosphodiesterase I) cDNA clones. The top line represents the sequence of PC-1 cDNA (7) with tick marks at 500-base-pair intervals. Five antibody-identified independent, overlapping clones are shown in addition to a clone (λ 1-2-2) isolated by DNA screening. The clone 1-2-2 containing the most 5′ sequence also contained ≈260 base pairs of unrelated sequence (dashed line). Sequence analysis revealed that translational termination codons are immediately present only four amino acids into this sequence, indicating it most likely represents a cloning artifact.

protein, PC-1. In fact, Table 1 shows that the anti-PC-1 antibodies recognized nucleotide pyrophosphatase/alkaline phosphodiesterase I, since active enzyme could be precipitated with this antiserum and protein A-Sepharose. In addition, this antiserum was used to analyze immunoblots of cell lysates from MOPC 315 cell lines known to have different levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity. We have shown that 315/P variant cells have a marked increase in nucleotide pyrophosphatase specific activity compared with wild-type MOPC 315/J cells (12), and levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity can be modulated in MOPC 315 cells with the glucocorticoid dexamethasone (14). The anti-PC-1 antiserum detected two protein bands (Fig. 3) with apparent molecular masses similar to the two previously identified forms of nucleotide pyrophosphatase/alkaline phosphodiesterase I. Furthermore, the staining intensities of these bands corresponded to the levels of enzymatic activity expressed in each cell line.

Correlation of PC-1 mRNA Expression and Nucleotide Pyrophosphatase/Alkaline Phosphodiesterase I Activity. The ability of dexamethasone to modulate the levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity in MOPC 315 cells was utilized to confirm further that the isolated PC-1 cDNA encoded the protein responsible for nucleotide pyrophosphatase/alkaline phosphodiesterase I activities. Total RNA was isolated from cells expressing various levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity and Northern analyses were performed with the E1A1 insert as hybridization probe. A 3.2-kilobase mRNA band was detected in all cases, and the intensities of the mRNA bands correlated perfectly with levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I activity in these cells (Fig. 4).

DISCUSSION

In this study we present evidence that the murine plasma cell differentiation antigen PC-1 has nucleotide pyrophosphatase/alkaline phosphodiesterase I activity. This conclusion is based primarily on data showing that antibodies to purified nucleotide pyrophosphatase/alkaline phosphodiesterase I specifically recognized recombinant PC-1 expression vector

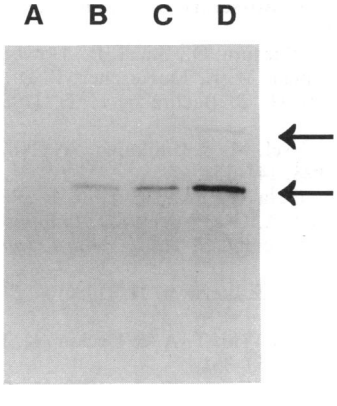

FIG. 3. Western immunoblot analysis of MOPC 315 cell lysates. Proteins from MOPC 315/J cells (lanes A and B) and variant 315/P cells (lanes C and D) were separated by SDS/PAGE, transferred to Immobilon-P, and analyzed with antiserum raised to PC-1 recombinant protein. Lysates analyzed in lanes B and D were obtained from cells incubated with 200 nM dexamethasone for 48 hr. Fifty micrograms of 1% Triton-soluble proteins was examined by Western immunoblot analysis. Alkaline phosphodiesterase I specific activities of cell extracts shown in lanes A–D were 0.12, 2.32, 6.24, and 18.84 μmol per hr per mg of protein, respectively. Arrows mark the positions of the 115-kDa and 220-kDa bands.

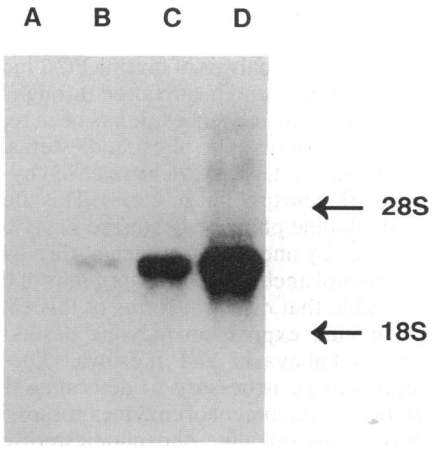

FIG. 4. Northern analysis of PC-1 (nucleotide pyrophosphatase/alkaline phosphodiesterase I) expression in MOPC 315 cells. Twenty micrograms of total RNA from MOPC 315/J cells (lanes A and B) or 315/P cells (lanes C and D) was electrophoresed in a formaldehyde/agarose gel and transferred to a nitrocellulose filter. RNA analyzed in lanes B and D was obtained from cells incubated with 200 nM dexamethasone for 48 hr. The blot was hybridized with the nick-translated 2.7-kilobase cDNA isolated from λ clone E1A1. Specific activities of alkaline phosphodiesterase I determined in cells used for RNA isolation shown in lanes A–D were 0.19, 1.36, 6.10, and 16.07 μmol per hr per mg of protein, respectively. Following prolonged exposure, the autoradiogram demonstrated a single band in lane A migrating with the major bands seen in lanes C–D. Migration of ribosomal RNAs is shown on the right.

clones and that other antibodies raised to a recombinant PC-1 fusion protein recognized this enzyme. Furthermore, in cell lines known to express different levels of nucleotide pyrophosphatase/alkaline phosphodiesterase I, the specific activity correlated with PC-1 mRNA expression. Finally, enzyme activity and PC-1 expression were coordinately modulated by the synthetic glucocorticoid dexamethasone. Together, these data indicate that the PC-1 gene encodes a nucleotide pyrophosphatase/alkaline phosphodiesterase I. That PC-1 is an enzyme is not surprising, since several human and murine lymphocyte differentiation antigens have been shown to have enzymatic activity. Although few differentiation antigens have been shown to be specifically expressed at late stages of B-cell development, recent studies have correlated the expression of another enzyme, alkaline phosphatase, with mouse (31) and rat (32) B-cell differentiation into immunoglobulin-secreting cells. Any functional relationship between alkaline phosphatase and nucleotide pyrophosphatase/alkaline phosphodiesterase I during B-cell maturation remains to be determined.

Analysis of the PC-1 cDNA sequence predicts certain structural features that are likely to be important for enzyme activity. For example, the presence of a potential consensus "EF-hand" domain (8) indicates the possible binding of divalent cations by the PC-1 protein. Indeed, mouse liver nucleotide pyrophosphatase/alkaline phosphodiesterase I has an obligate divalent cation requirement for enzymatic activity (9). Consistent with these reports, we have found that EDTA completely abolishes murine plasmacytoma nucleotide pyrophosphatase/alkaline phosphodiesterase I activity (unpublished data). The deduced amino acid sequence of PC-1 also contains eight tripeptide consensus sequences for potential N-linked glycosylation (Asn-Xaa-Thr/Ser). We have not determined exact oligosaccharide numbers or precise structures, but nucleotide pyrophosphatase/alkaline phosphodiesterase I does contain N-linked oligosaccharide moieties as documented by its interaction with lectin affinity

columns and its altered electrophoretic migration after N-Glycanase digestion.

The cDNA sequence analysis of murine PC-1 indicates that it is a type II membrane protein anchored through N-terminal hydrophobic amino acid sequences. It has been reported that varying percentages of alkaline phosphodiesterase I activity in some, but not all, rat tissues can be released by phosphatidylinositol-specific phospholipase C (33). This indicates that some forms of alkaline phosphodiesterase I may be attached to cell membranes by another mechanism, through glycosylphosphatidylinositol anchors at the C terminus of the protein. Thus, it is possible that different forms of this enzyme exist and that isoenzyme expression may be tissue-specific as suggested by Nakabayashi and Ikezawa (33). However, further studies will be necessary to determine directly the mode of membrane attachment of enzymes possessing nucleotide pyrophosphatase/alkaline phosphodiesterase I activity in different tissues.

Although the physiological role that nucleotide pyrophosphatase/alkaline phosphodiesterase I plays in the differentiating B cell has not been determined, a type II orientation may be functionally significant. Subcellular localization studies analyzing rat liver cells have demonstrated enzymatic activity in the rough endoplasmic reticulum as well as the plasma membrane (34). With the short N-terminal region exposed to the cytosol, a relatively large C-terminal catalytic domain would be oriented toward the lumen of the rough endoplasmic reticulum and Golgi apparatus. We have previously speculated (12) that the nucleotide pyrophosphatase activity of nucleotide pyrophosphatase/alkaline phosphodiesterase I regulates the availability of nucleotide sugar precursors at the site of oligosaccharide synthesis. Evidence in support of this hypothesis included the finding of diminished [^{3}H]mannose incorporation into the larger lipid-linked oligosaccharide precursors of the 315/P cells possessing high nucleotide pyrophosphatase specific activities (12). PC-1 (nucleotide pyrophosphatase/alkaline phosphodiesterase I) does not have significant sequence homology with other known proteins, but it is of interest that it shares the type II membrane protein domain structure in common with many glycosyltransferases (35). These enzymes are essential for the developmentally regulated expression of specific oligosaccharide structures. Since competition of terminal glycosyltransferases for common substrates is an important mechanism for determining oligosaccharide sequences (36), nucleotide pyrophosphatase may influence glycosylation by regulating glycosyltransferase substrate availability not only in the rough endoplasmic reticulum but also at other subcellular sites of oligosaccharide processing. Additional studies utilizing the cloned cDNA for nucleotide pyrophosphatase/alkaline phosphodiesterase I will further help to define the function of this enzyme and establish its relationship with the type II glycosyltransferases.

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