FEBS Letters 408 (1997) 52–56 FEBS 18537

Molecular heterogeneity of the cDNA encoding a 74-kDa regulatory subunit (B″ or δ) of human protein phosphatase 2A

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Received 7 March 1997

Abstract Two cDNAs for possible splicing variants of a 74-kDa regulatory subunit (B″ or δ) of human protein phosphatase 2A, were isolated. These variants were identified from human cerebral cortex by library screening and PCR, and designated δ1 and δ3 isoforms, while the previously reported isoform [Tanabe et al. (1996) FEBS Lett. 379, 107–111] was designated δ2. Compared with the δ2 isoform, the δ1 isoform contained a 32-residue insertion beginning at residue 84, and consisted of 602 amino acids in all. The δ3 isoform lacked a 74-residue sequence corresponding to residues 1083 of the δ2 isoform, and consisted of 496 amino acids. Using isoform-specific antipeptide antisera, the 74-kDa subunit (B″ or δ) originally purified from human erythrocytes was identified as the δ1 isoform.

Key words: Protein phosphatase 2A; 74-kDa regulatory subunit (B″ or δ); Human erythrocyte; Human cerebral cortex; Splicing variant; B′ subunit

1. Introduction

Protein phosphatase 2A (PP2A), one of the four major protein serine/threonine phosphatases, is found in all eukaryotes, and plays crucial roles in the regulation of many cellular processes including metabolism, cell cycle, DNA replication, transcription, translation, and viral transformation [1,2]. PP2A holoenzymes with either a heterodimeric or a heterotrimeric subunit structure have been purified from various mammalian tissues and organisms. The heterodimeric structure is composed of a 32–41-kDa catalytic C subunit and a 60–69-kDa regulatory A subunit. The AC heterodimer is a common component of all PP2A holoenzymes identified to date. The trimeric holoenzyme contains an additional variable regulatory subunit of either 51–58-kDa B [1,3], 54-kDa B′ [4], 72-kDa PR72 [5], or 74-kDa δ (also called B″) [6]. Molecular cloning of these subunits has revealed that these variable subunits are classified into three unrelated families, namely, B [7–10], PR72 [11], and B′ families. The B′ family was identified most recently, and found to be encoded by five distinct genes that produce several splicing variants [12–17].

From human erythrocyte cytosol, we purified three forms of PP2A whose subunit structures are $\alpha_1\beta_1$, $\alpha_1\beta_1\gamma_1$, and $\alpha_1\beta_1\delta_1$, where α corresponds to the catalytic C subunit, and β, γ, and δ correspond to the A, B, and B″ regulatory subunits, respectively [6]. Based on partial amino acid sequences of the δ (B″) subunit, its cDNA was isolated from human cerebral cortex and bone marrow cDNA libraries [12]. Recent molecular cloning of the B′ [14,15], B56 [13,17], and PR61 [16] subunits of PP2A has revealed that these subunits and the δ (B″) subunit have strong sequence similarity, especially in the central 400-amino acid region of the molecules with more than 70% identity, and comprise a new family of the regulatory subunit of PP2A, the B′ family. The predicted primary structure of the δ (B″) subunit is most similar to that of human B56δ among the family members, and different only by a 32-amino acid insertion in B56δ near the N terminus [12,17]. The predicted primary structure of the δ (B″) subunit is also similar to rabbit B′γ, which contains the same 32-amino acid insertion and a unique N-terminal 4-residue sequence instead of the N-terminal 20-residue sequence in the δ subunit [12,14]. Comparison of the nucleotide sequences of the cDNAs for the human δ (B″), B56δ, and rabbit B′γ subunits suggests that they may be generated by alternative splicing. The variable regulatory subunits of PP2A have been suggested to play the roles of regulating the activity, and determining the substrate specificity and the intracellular localization of PP2A [1,2]. Therefore, characterization of each regulatory subunit is necessary for better understanding of the function and regulation of PP2A.

In this study, we identified two isoforms of the δ (B″) subunit possibly generated by alternative splicing, designated δ1 and δ3, while the previously reported isoform [12] was designated δ2. Compared with the δ2 isoform, the δ1 isoform contained a 32-amino acid insertion near the N terminus, and was identical with B56δ [17]. The δ3 isoform lacked a 74-amino acid sequence found in the δ2 isoform. Using specific antisera raised against peptides which have the δ1-specific insertion sequence and the N-terminal sequence common to the δ1/δ2 isoforms, the 74-kDa δ (B″) subunit originally purified from human erythrocytes was identified as the δ1 isoform.

2. Materials and methods

2.1. Amplification of δ cDNAs by PCR

Initial amplification of the 5′ end fragments of the δ cDNAs from HeLa cell cDNA and human cerebral cortex cDNA (Clontech) was achieved by PCR with δ-specific primers, 5′-GCGGATCCATGCCCTATAAACTGAAA-3′ (forward primer, DF1) and 5′-TGAAAACATGGTGACAGC-3′ (reverse primer, DR1) corresponding to nucleotides 78–95 and 492–509, respectively (Fig. 1). The nucleotide numbers are based on the δ2 cDNA sequence reported previously [12]. The reactions were carried out in a volume of 50 μl containing 20 mM Tris-HCl, pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, 0.1 mg/ml bovine serum albumin, 0.2

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Abbreviations: ABRPP2A, protein phosphatase 2A; PCR, polymerase chain reaction; KLH, keyhole limpet hemocyanin; MBS, *m*-maleimidobenzoyl-*N*-hydoxysuccinimide ester; PAGE, polyacrylamide gel electrophoresis; bp, base pair; kb, kilobase

The nucleotide sequences reported in this paper have been submitted to the DDBJ, EMBL and GenBank databases with accession numbers AB000634 (δ1) and AB000635 (δ3).

PII S0014-5793(97)00392-X

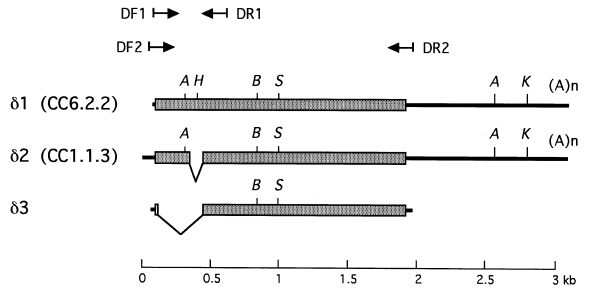

Fig. 1. Structures of cDNAs for three isoforms of the human δ (B″) subunit of PP2A. The cDNAs for the δ1 and δ3 isoforms were isolated from human cerebral cortex by library screening and PCR. The cDNA for the δ2 isoform was previously isolated from the human cerebral cortex cDNA library [12]. The open reading frames are shown as boxes, and poly(A) tails are shown as (A)n. The angled lines in the δ2 and δ3 cDNAs indicate deletions. The arrows indicate the positions and directions of the primers used for PCR. The restriction sites for *Apa*I (A), *Hin*dIII (H), *Bam*HI (B), *Sac*I (S), and *Kpn*I (K) are indicated.

mM deoxynucleoside triphosphates, 0.2 μM forward and reverse primers, 1.25 units of Taq polymerase (Boehringer), and 1.25 units of Taq Extender PCR Additive (Stratagene) with 35 amplification cycles, each consisting of 30 s denaturation at 95°C, 30 s annealing at 54°C, and 90 s extension at 72°C. Amplified fragments were separated in ethidium bromide-stained 1.5% agarose gel, purified with DEAE-cellulose membrane (Schleicher and Schuell), and cloned into the pUC19 plasmid for sequence analysis.

Isolation of the whole coding region of the δ3 cDNA from human cerebral cortex cDNA was achieved by PCR with another set of δ-specific primers, 5′-GCTCTAGAGCCGGAGCGGGGCCGCAGGA-3′ (forward primer, DF2) and 5′-GCGAATTCGGAGGGCCAGGGCAGTGTCCCA-3′ (reverse primer, DR2) corresponding to nucleotides 30–51 and 1832–1853, respectively, in the untranslated regions of the δ2 cDNA (Fig. 1). The reaction was carried out in the same mixture as described above with 35 amplification cycles, each consisting of 30 s denaturation at 94°C, and 3 min annealing with extension at 68°C. Amplified fragments were separated in 1.0% agarose gel, and cloned into the pUC19 plasmid for sequence analysis. The sequences were determined on both strands using the AutoRead sequence kit (Pharmacia) and A.L.F. DNA Sequencer II (Pharmacia). The underlined sequences in the primers are restriction sites introduced for cloning the PCR products into plasmids.

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   1  CCGGACGGGCCGAGATGCCCTATAAACTGAAAAAGGAGAAGGAGCCCCCCAAGGTTGCCAAATGCACAGCCAAGCCTAGCAGCTCGGGCAAGGATGGTGGAGGC  104
   1            M  P  Y  K  L  K  K  E  K  E  P  P  K  V  A  K  C  T  A  K  P  S  S  S  G  K  D  G  G  G      30

 105  GAGAACACTGAGGAGGCCCAGCCGCAGCCCCAGCCCCAGCCCCAGCCCCAAGCCCAGTCTCAGCCACCGTCATCCAACAAGCGTCCCAGCAATAGCACGCCGCCC  209
  31  E  N  T  E  E  A  Q  P  Q  P  Q  P  Q  P  Q  P  Q  P  Q  A  Q  S  Q  P  P  S  S  N  K  R  P  S  N  S  T  P  P   65

 210  CCCACGCAGCTCAGCAAAATCAAGTACTCAGGGGGGCCCCAGATTGTCAAGAAGGAGCGACGGCAAAGCTCCTCCCGCTTCAACCTCAGCAAGAATCGGGAGCTG  314
  66  P  T  Q  L  S  K  I  K  Y  S  G  G  P  Q  I  V  K  K  E  R  R  Q  S  S  S  R  F  N  L  S  K  N  R  E  L  100

 315  CAGAAGCTTCCTGCCCTGAAAGATTCGCCAACCCAGGAGCGGGAGGAGCTGTTTATCCAGAAGCTACGCCAGTGCTGTGTCCTCTTTGACTTCGTGTCAGACCCA  419
 101  Q  K  L  P  A  L  K  D  S  P  T  Q  E  R  E  E  L  F  I  Q  K  L  R  Q  C  C  V  L  F  D  F  V  S  D  P  135

 420  CTCAGTGACCTCAAATTCAAGGAGGTGAAGCGGGCAGGACTCAACGAGATGGTGGAGTACATCACCCATAGCCGTGATGTTGTCACTGAGGCCATTTACCCTGAG  524
 136  L  S  D  L  K  F  K  E  V  K  R  A  G  L  N  E  M  V  E  Y  I  T  H  S  R  D  V  V  T  E  A  I  Y  P  E  170

 525  GCTGTCACCATGTTTTCAGTGAACCTCTTCCGGACGCTGCCACCTTCATCGAATCCCACAGGGGCTGAGTTTGACCCAGAGGAAGATGAGCCCACCCTGGAAGCT  629
 171  A  V  T  M  F  S  V  N  L  F  R  T  L  P  P  S  S  N  P  T  G  A  E  F  D  P  E  E  D  E  P  T  L  E  A  205

 630  GCTTGGCCACATCTCCAGCTCGTGTATGAGTTCTTCTTACGTTTCCTTGAGTCTCCTGATTTCCAGCCAAACATAGCCAAGAAGTACATCGACCAGAAGTTTGTA  734
 206  A  W  P  H  L  Q  L  V  Y  E  F  F  L  R  F  L  E  S  P  D  F  Q  P  N  I  A  K  K  Y  I  D  Q  K  F  V  240

 735  CTTGCTCTCCTAGACCTATTTGACAGTGAGGATCCTCGAGAGCGGGACTTCCTCAAGACCATTTTGCATCGCATCTATGGCAAGTTTTTGGGGCTCCGGGCTTAT  839
 241  L  A  L  L  D  L  F  D  S  E  D  P  R  E  R  D  F  L  K  T  I  L  H  R  I  Y  G  K  F  L  G  L  R  A  Y  275

 840  ATCCGTAGGCAGATCAACCACATCTTCTACAGGTTCATCTACGAGACGGAGCATCACAACGGGATTGCTGAGCTCCTGGAGATCCTGGGCAGCATCATCAATGGC  944
 276  I  R  R  Q  I  N  H  I  F  Y  R  F  I  Y  E  T  E  H  H  N  G  I  A  E  L  L  E  I  L  G  S  I  I  N  G  310

 945  TTTGCCCTGCCCCTTAAAGAAGAGCACAAGATGTTCCTCATCCGTGTCCTACTTCCCCTTCACAAGGTCAAGTCCCTGAGTGTCTACCACCCTCAGCTGGCATAC 1049
 311  F  A  L  P  L  K  E  E  H  K  M  F  L  I  R  V  L  L  P  L  H  K  V  K  S  L  S  V  Y  H  P  Q  L  A  Y  345

1050  TGTGTGGTACAATTCCTGGAGAAGGAGAGCAGTCTGACTGAGCCGGTAATTGTGGGACTTCTCAAGTTTTGGCCCAAGACCCACAGCCCCAAGGAGGTGATGTTC 1154
 346  C  V  V  Q  F  L  E  K  E  S  S  L  T  E  P  V  I  V  G  L  L  K  F  W  P  K  T  H  S  P  K  E  V  M  F  380

1155  TTGAATGAGCTGGAGGAGATTCTGGACGTCATTGAACCTTCTGAGTTCAGCAAAGTGATGGAACCCCTCTTCCGCCAGCTGGCCAAGTGTGTCTCTAGCCCCCAT 1259
 381  L  N  E  L  E  E  I  L  D  V  I  E  P  S  E  F  S  K  V  M  E  P  L  F  R  Q  L  A  K  C  V  S  S  P  H  415

1260  TTCCAGGTGGCAGAGCGTGCTCTCTATTACTGGAACAATGAGTACATCATGAGCCTGATAAGTGACAATGCTGCCCGAGTCCTCCCCATCATGTTCCCTGCACTC 1364
 416  F  Q  V  A  E  R  A  L  Y  Y  W  N  N  E  Y  I  M  S  L  I  S  D  N  A  A  R  V  L  P  I  M  F  P  A  L  450

1365  TACAGGAACTCCAAGAGCCACTGGAACAAGACAATCCATGGACTGATCTATAATGCCCTGAAGTTGTTTATGGAAATGAATCAGAAGCTGTTTGATGACTGCACA 1469
 451  Y  R  N  S  K  S  H  W  N  K  T  I  H  G  L  I  Y  N  A  L  K  L  F  M  E  M  N  Q  K  L  F  D  D  C  T  485

1470  CAACAATACAAGGCAGAGAAGCAGAAGGGCCGGTTCCGAATGAAGGAAAGGGAAGAGATGTGGCAAAAAATCGAGGAGCTGGCCCGGCTTAATCCCCAGTATCCC 1574
 486  Q  Q  Y  K  A  E  K  Q  K  G  R  F  R  M  K  E  R  E  E  M  W  Q  K  I  E  E  L  A  R  L  N  P  Q  Y  P  520

1575  ATGTTCCGAGCCCCTCCACCACTGCCCCCTGTGTACTCGATGGAGACAGAGACCCCCACAGCTGAGGACATCCAGCTTCTGAAGAGGACTGTGGAGACTGAGGCT 1679
 521  M  F  R  A  P  P  P  P  L  P  P  V  Y  S  M  E  T  E  T  P  T  A  E  D  I  Q  L  L  K  R  T  V  E  T  E  A  555

1680  GTTCAGATGCTAAAAGACATCAAGAAGGAGAAAGTGCTGCTGCGGAGGAAGTCGGAGCTGCCCCAGGACGTGTACACCATCAAGGCACTGGAGGCGCACAAGCGG 1784
 556  V  Q  M  L  K  D  I  K  K  E  K  V  L  L  R  R  K  S  E  L  P  Q  D  V  Y  T  I  K  A  L  E  A  H  K  R  590

1785  GCGGAAGAGTTCCTAACTGCCAGCCAGGAGGCTCTCTGACCCCTCACGTTCCTACCACAGGGCCACAGCCCACACAGCCCTGGGACACTGCCCTGGCCCTCCATA 1889
 591  A  E  E  F  L  T  A  S  Q  E  A  L  *                                                                    602

1890  CTCTGCTCCCTACTGGCTGTCTTGGGGGAAGGCAGCGCCTCTCTAGCTACTCAAGGGAGGGGGATGTGGGCACTTGAAGCAGGGACACCCACAGAATGGTCCCTC 1994
1995  TTCTCCCCAAAAGGTGTTCATGCCTCCCTGTGGCTAGTACAGGCTGAGCACTAAGATGCTTAGTGCTCAGACAACCTGGGGATGCCTGTCCCCTACCTGCTCCTC 2099
2100  ACCCACAGCTACCTGAGGCTGCTCTGAGAAGTACACACAGGAATACATACGCTCCTCTATTCTTCCCTTCATCCTCATTTGAACGCCAGGTATCTCCCCTCCTCT 2204
2205  CTCTCCCCTGCAG                                                                                              2217
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Fig. 2. Nucleotide and deduced amino acid sequences of the δ1 cDNA. The δ2 cDNA lacked the region indicated with the double underline. The δ3 cDNA lacked the region indicated with the single and double underlines. The asterisk represents the stop codon. The dotted lines indicate the sequences used for production of the antipeptide antisera. Potential phosphorylation sites for protein kinase A (△) and protein kinase C (▲) are indicated [18].

2.2. Production of antipeptide antisera

The N-terminus peptide, MPYKLKKEKEPPKVC, consisting of a sequence from residues 1–14 of the δ1/δ2 isoforms and an additional cysteine residue (underlined), and the insertion peptide, CKNRELQKLPALKDSPT, consisting of a sequence from residues 96–111 of the δ1 isoform and an additional cysteine residue (underlined), were synthesized (TANA Laboratories). The peptides were coupled to KLH through the cysteine residue using MBS as a coupling reagent. Sixteen milligrams of KLH was dissolved in 1 ml of 10 mM sodium phosphate buffer, pH 7.2, and then mixed with 2.8 mg of MBS dissolved in 10 μl of dimethylformamide. The reaction mixture was incubated for 30 min at room temperature with stirring, and passed through a Sephadex G-25 column (40×1.0 cm). The protein peak was collected, and one-tenth of the solution (0.7 ml) was mixed with 0.35 ml of 0.2 M sodium phosphate buffer, pH 7.2 and 1 mg of the synthetic peptide dissolved in 0.5 ml of distilled water. The reaction mixture was incubated in N_2 gas for 3 h at room temperature with continuous rotation. The KLH-peptide conjugates (0.2 mg peptide) were emulsified in Freund's complete adjuvant, and then injected subcutaneously into New Zealand White rabbits. Three additional booster injections of KLH-peptide conjugates (0.1 mg peptide) emulsified in Freund's incomplete adjuvant were given at 2 to 4-week intervals. Enzyme-linked immunosorbent assay was used to screen for antipeptide antisera using the peptide antigens. Sera were collected 2 weeks after the third booster injections.

2.3. Bacterial expression and Western blot analysis

The δ1 and δ2 isoforms were expressed in bacteria using the pKK233-2 vector (Clontech). The pKK233-2-δ2 plasmid carrying the protein coding sequence of the δ2 cDNA was described previously [12]. A pKK233-2-δ1 plasmid carrying the δ1 cDNA was constructed by replacing a 570-bp *Apa*I-*Sac*I fragment in pKK233-2-δ2 with a 666-bp *Apa*I-*Sac*I fragment in the δ1 cDNA (Fig. 1). The constructs were verified by sequencing. *Escherichia coli* DH5α cells were transformed with these constructs and grown in Luria-Bertani medium supplemented with 100 μg/ml ampicillin at 37°C until OD_{600} was 0.50. The bacteria were further incubated for 2 h with 1 mM isopropylthio-β-D-galactoside. From 3 ml of the culture, cells were collected by centrifugation, lysed in 0.3 ml of SDS-sample buffer (10 mM Tris-HCl, pH 7.8, 3% SDS, 5% glycerol, 2.5 mM dithiothreitol, 0.0167% bromophenol blue), and then heated in a boiling water bath for 3 min. After centrifugation at 11 000×g for 1 min, 27 μl of the supernatants was mixed with 3 μl of 0.63 M iodoacetamide, and then subjected to SDS-PAGE with 10% polyacrylamide gel. After electrophoresis, proteins in the gels were electrophoretically transferred to polyvinylidene difluoride membranes (Millipore) and then probed with antibodies as described previously [12]. The rabbit antipeptide antisera diluted 8000-fold were used as the primary antibody, and goat anti-rabbit IgG antibody conjugated to horseradish peroxidase (Kirkegaard and Perry) was used as the secondary antibody. Immunoreactive proteins on the membranes were detected with an enhanced chemiluminescence system (Amersham).

3. Results and discussion

Based on partial amino acid sequences of a 74-kDa regulatory subunit (B″ or δ) of human erythrocyte PP2A, the cDNA was isolated from human cerebral cortex and bone marrow cDNA libraries [12]. The differences in the predicted primary structure near the N terminus between the human δ (B″) subunit and its possible rabbit counterpart, the B′γ subunit [14], prompted us to search for other variants of the δ (B″) subunit in the cDNA clones already isolated from the human cerebral cortex library. Of the 29 δ (B″) cDNA clones obtained by screening 1.3×10^6 plaques, 20 clones showed the same restriction maps as that of the clone CC1.1.3 reported previously [12]. However, 9 clones contained inserts with a different restriction map near 5′ ends (Fig. 1). One of these clones, CC6.2.2, about 3.0 kb long, was subjected to sequence analysis. The nucleotide sequence of the whole coding region was determined, and shown to be different from CC1.1.3 only by an in-frame 96-bp insertion found in CC6.2.2 near the 5′ end (Fig. 2). This newly identified clone appeared to be an alternative splicing variant of the δ (B″) subunit, and was designated δ1, while the previously identified cDNA was designated δ2. The δ1 cDNA contained a 1806-bp open reading frame encoding a protein of 602 amino acids with a calculated molecular weight of 69 947 (Fig. 2). The deduced primary structure of the δ1 isoform was different from that of δ2 only by a 32-amino acid insertion in δ1 beginning at residue 84. The nucleotide sequence of the δ1 cDNA was identical with that of human B56δ reported recently [17], except one base substitution in the coding region without any difference in the predicted primary structure. The rabbit B′γ subunit is nearly identical with the δ1 isoform except that B′γ contains a unique N-terminal 4-residue sequence instead of a 20-residue sequence of δ1, and appears to be another splicing variant of the δ subunit [14].

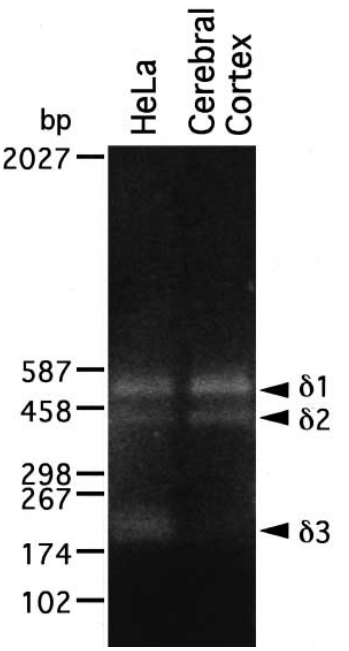

Fig. 3. Detection of δ cDNAs by PCR. Human HeLa cell and cerebral cortex cDNAs were subjected to PCR with the δ-specific primers (DF1 and DR1) as described in Section 2. Five microliters of each reaction mixture was resolved on a 1.5% agarose gel stained with ethidium bromide (0.5 μg/ml). Positions of the PCR products are indicated with the arrow heads. The migration of molecular size standards (bp) is indicated to the left of the panel.

To confirm the expression of two different δ mRNAs, PCR was performed with δ-specific primers, DF1 and DR1, corresponding to the sequences common to the δ1 and δ2 cDNAs, using human HeLa cell and cerebral cortex cDNAs as templates (Fig. 1). These primers were designed to sandwich the δ1-specific insertion and to yield products of different sizes from the δ1 and δ2 cDNAs. Fragments of about 540 bp and 440 bp, which coincide with the expected products from the δ1 and δ2 mRNAs, respectively, were amplified from both templates (Fig. 3). Identification of these fragments with the δ1 and δ2 cDNAs was verified by subcloning and sequencing (data not shown). In addition to them, a smaller fragment of about 220 bp was amplified from both templates. This fragment was also cloned into the pUC19 plasmid, and shown to

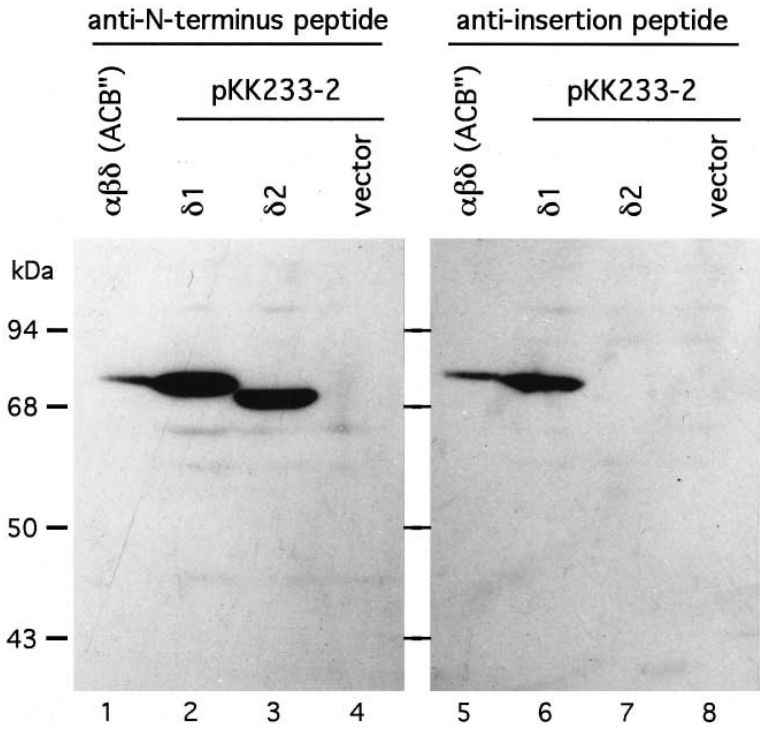

Fig. 4. Immunoblotting of bacterially expressed δ1 and δ2 isoforms and the 74-kDa δ (B″) subunit from human erythrocytes using the antipeptide antisera. Immunoblots are shown of extracts from bacteria transformed by the pKK233-2 plasmids carrying the δ1 cDNA (pKK233-2-δ1, lanes 2 and 6) and the δ2 cDNA (pKK233-2-δ2, lanes 3 and 7), and the vector only (lanes 4 and 8). Lanes 1 and 5 contain 50 ng of human erythrocyte $\alpha_1\beta_1\delta_1$ (ACB″). The immunoblots were probed with the antisera against the N-terminus peptide (lanes 1–4) and the insertion peptide (lanes 5–8). The migration of molecular mass standards (kDa) is shown to the left of the panels.

be identical with the δ1 and δ2 cDNAs except a deletion in the fragment after 27th nucleotide from the initiation codon. This 220-bp cDNA fragment seemed to be another splicing variant of the δ subunit, and was designated δ3 (Figs. 1 and 2).

In order to isolate the whole coding region of the δ3 cDNA, we synthesized another set of δ-specific primers, DF2 and DR2, corresponding to the 5′ and 3′ untranslated regions of the δ2 cDNA, respectively (Fig. 1). By PCR using these primers, the δ3 cDNA of 1.6 kb, containing a sequence identical with the 220-bp fragment, was isolated from the human cerebral cortex cDNA. The complete nucleotide sequence of the δ3 cDNA was determined with three independent clones, and shown to contain a 1488-bp open reading frame encoding a protein of 496 amino acids with a calculated molecular weight of 58 415. The δ3 cDNA was different from the δ1 and δ2 cDNAs only by the deletion found in the 220-bp fragment (Figs. 1 and 2). The predicted primary structure of the δ3 isoform showed a deletion of residues 10–115 of the δ1 isoform (Fig. 2).

Comparison of the nucleotide sequences of the δ1, δ2, and δ3 cDNAs suggests that they may be generated by alternative splicing that joins different splice donor sites to a common acceptor site. The predicted amino acid sequences of the δ2 and δ3 isoforms lacked 32- and 106-amino acid sequences found in the δ1 isoform, respectively. In these regions, a cluster of consensus phosphorylation sites for protein kinases A and C [18] and a unique glutamine-proline repeat [12] were identified (Fig. 2). The 74-kDa δ (B″) subunit in a holoenzyme was indeed phosphorylated by protein kinase A in vitro, resulting in a slight increase in phosphatase activities toward phosphorylated histones and phosphorylase [19]. The epitope-tagged overexpressed B56δ was also shown to be phosphorylated in vivo [17]. These results suggest that the function of the δ (B″) subunit might be regulated in response to signals transduced by protein phosphorylation-dephosphorylation.

To determine the isoform of the 74-kDa δ (B″) subunit originally purified from human erythrocytes, two isoform-specific antipeptide antisera were raised against synthetic peptides corresponding to the 16-residue sequence in the δ1-specific 32-residue insertion and the N-terminal 14-residue sequence common to the δ1 and δ2 isoforms. By Western blot analysis, the bacterially expressed δ1 isoform was recognized by both antisera, and the bacterially expressed δ2 isoform reacted only with the antiserum against the N-terminus peptide (Fig. 4). The 74-kDa δ (B″) subunit was recognized by both antisera, and showed exactly the same electrophoretic mobility as the δ1 isoform expressed in bacteria. The bacterially expressed δ2 isoform showed slightly faster electrophoretic mobility than the 74-kDa δ (B″) subunit. These data indicate that the 74-kDa δ (B″) subunit originally purified from human erythrocytes corresponds to the δ1 isoform containing the 32-amino acid insertion.

By this study, the number of possible splicing variants of the δ subunit was brought to four, counting the rabbit B′γ subunit [12,14,17]. The total number of the B′ family isoforms has come to at least thirteen [12–17]. The diversity of the regulatory subunits may provide a molecular basis for so many important roles of PP2A in different cellular processes.

Acknowledgements: We thank Dr. Shigetada Nakanishi (Kyoto University) for providing the human cerebral cortex cDNA library, and Dr. Takuya Shimamoto (Osaka University) for providing HeLa cell

cDNA. We thank Mieko Kawamura for her excellent secretarial assistance, and Ryoko Takemoto and Aki Ikeda for their skilful technical assistance. We also thank Research Center for Molecular Medicine at Hiroshima University for the DNA sequencer. This work was supported in part by Grants-in-Aid for Cancer Research and Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (1990–1996).

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