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Isolation and sequence determination of cDNA encoding P2 protein of human peripheral myelin*

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Summary: **A full length cDNA of P2 protein of peripheral myelin has been isolated from a cDNA library of human fetus spinal cord. The clone is 2150 base pairs (bp) in length and contains a 393 bp open reading frame encoding a polypeptide of 131 residues. The deduced amino acid sequence is highly homologous to P2 protein from other species.**

Myelin is a multilamellar compacted membrane structure that surrounds and insulates the axon, facilitating the conduction of nerve impulses. Schwann cells are responsible for myelin formation in the peripheral nerve. The P2 protein is a small basic protein found in peripheral myelin. The primary structure of P2 protein has been directly determined by protein sequencing(1,2,3). Its amino acid sequence is similar to a family of fatty acid binding proteins(4) and bovine P2 protein is shown to have lipid binding activities(5). Myelin is composed of more lipids(70 %) and less proteins(30 %). With this lipid binding activities, P2 protein may have some important role in the organization of compact myelin. The cDNA has been isolated from rabbit(4) and genomic structure has been determined in mouse(6). The cDNA encoding human P2 protein has not been isolated; its information is necessary for the

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study of genetic disorders. In the present study we report the isolation of a cDNA encoding the entire human P2 protein from human fetus spinal cord library.

Materials and Methods

Materials Restriction nucleases and DNA-modifying enzymes were purchased from Takara Shuzo (Kyoto,Japan). Radiolabeled nucleotides were obtained from New England Nuclear (Boston,MA). Oligonucleotide primers were synthesized on an Applied Biosystems 381A DNA synthesizer.

Screening of cDNA Library A lambda gt11 library from human fetus spinal cord(7) was screened with a radiolabelled cDNA of rabbit P2 protein(4). Filters were hybridized with ^{32}P-labelled probe overnight at 65°C in 6X SSC containing 5X Denhardt's solution, 10 % dextran sulfate, 10 mM EDTA, 0.5 % SDS, and 100 μg/ml salmon sperm DNA. They were then washed at room temperature in 2X SSC containing 0.1 % SDS, followed by washing for 30 min at 65°C in 2X SSC containing 0.1 % SDS, and exposed to X-ray film. Twenty two positive clones were isolated from 1 x 10^6 plaques. Three of them were more than 2000 bp long.

DNA Sequencing DNA inserts liberated by digestion with EcoRI were isolated, purified and subcloned in pUC 19 using standard procedures. Nucleotide sequences were determined by the dideoxy method(8). The nucleotide sequences, the deduced primary structures and homologies were analyzed using a computer program, DNASIS(Hitachi Software Engineering Co., Ltd.).

Results and Discussion

Of the three clones that were isolated, one was 2200 bp long and the other two were 2100 and 2000 bp. The 2200 bp clone, A2h, was fully sequenced and shown in Fig. 1 ; the other two clones were partially sequenced and these sequences were found to be present within the fully sequenced clone except ^{76}C to T. Clone A2h is 2150 bp in length and has a 393 bp open reading frame encoding a polypeptide of 131 residues and 5 bp of the poly(A) tail at the 3' end. The nucleotide sequence of the human P2 protein is 59 % identical to that of rabbit P2 protein(4). The deduced amino acid sequence coincides with the sequence determined directly by protein sequencing except for ^{98}Asp to Asn and ^{110}Asn to Asp. The change in the nucleotide ^{76}C to T dose not change the amino acid and seems to be polymorphism. The amino acid sequence shows 95 % homology to rabbit(1) and 87 % to mouse P2 protein(6) (Fig.2). P2 protein may be important to make a compact myelin sheath in the peripheral nerve together with other proteins like P0 protein. The few interspecies amino acid variations indicate a quite conservative evolution of the P2 protein.

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   1    C GCT TAG AAC TGT GTT GAG CTC TCA CCC ATC ACG ATG AGC AAC AAA      46
   1                                                  Ser Asn Lys       3

  47  TTC CTG GGC ACC TGG AAA CTT GTC TCT AGC GAG AAC TTT GAC GAT TAC      94
   4  Phe Leu Gly Thr Trp Lys Leu Val Ser Ser Glu Asn Phe Asp Asp Tyr      19

  95  ATG AAA GCT CTG GGT GTG GGG TTA GCC ACC AGA AAA CTG GGA AAT TTG     142
  20  Met Lys Ala Leu Gly Val Gly Leu Ala Thr Arg Lys Leu Gly Asn Leu      35

 143  GCC AAA CCC ACT GTG ATC ATC AGC AAG AAA GGA GAT ATT ATA ACT ATA     190
  36  Ala Lys Pro Thr Val Ile Ile Ser Lys Lys Gly Asp Ile Ile Thr Ile      51

 191  CGA ACT GAA AGT ACC TTT AAA AAT ACA GAA ATC TCC TTC AAG CTA GGC     238
  52  Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly      67

 239  CAG GAA TTT GAA GAA ACC ACA GCT GAC AAT AGA AAG ACC AAG AGC ATC     286
  68  Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn Arg Lys Thr Lys Ser Ile      83

 287  GTA ACC CTG CAG AGA GGA TCA CTG AAT CAA GTG CAG AGA TGG GAT GGC     334
  84  Val Thr Leu Gln Arg Gly Ser Leu Asn Gln Val Gln Arg Trp Asp Gly      99

 335  AAA GAG ACA ACC ATA AAG AGA AAG CTA GTG AAT GGG AAA ATG GTA GCG     382
 100  Lys Glu Thr Thr Ile Lys Arg Lys Leu Val Asn Gly Lys Met Val Ala     115

 383  GAA TGT AAA ATG AAG GGC GTG GTG TGC ACC AGA ATC TAT GAG AAG GTC     430
 116  Glu Cys Lys Met Lys Gly Val Val Cys Thr Arg Ile Tyr Glu Lys Val     131

 431  TGA AAA ATC ATT TCT TCA TTG AAG TGG CTT TTT ATC ATT TAA TGA TGG     478
 132  ***                                                              132

 479  AAA TCA ATT GCT TCC ATT GAC AAA ACT GAA TAC ACT GCA AAT ATT TGT     526
 527  TTT TGC TTT TGT CTT AAT ATA TCA GAT ATG CAA AGG CCT AAA CTG AGA     574
 575  ATT AAT CTA AAA GTC AGT GTT ATT TAA ACA TTT TCA ATG TGC ATG CAT     622
 623  GTC ATT ATT ACA TCA AAG CAT ATA TAT TGG CCA GAC ACA AAC AGT TGA     670
 671  TGA TGT CAT TCA ATT AAC TAC AAA ATT CTA ATC TAT GTT GAA CTT TGT     718
 719  ATA CTT GAA ATG ATA ATA AAA AGG ATA TAA TTT CTT AGT AAA ATG AAA     766
 767  TCA AAG TAT TGA TCA GGG TAG CAA ACT CAA ATG CTG ACA GGG GCC AGA     814
 815  GGA GAT ATG GGG AAG GAG CAT CAG AAA TGA GGC AAG CTA GGA GAA TGG     862
 863  GCT ATT ATA ATG TAA AGA ATT GTA GTC TCA GTT AAA AGG GGT AGC CTC     910
 911  TAC TCC AGC CAA CAT TTT AAA ATT AAT GGA TAA TTT ATA GAC AGA TAA     958
 959  ATT TAT AGA CAG TTA AGT AAA AAT GGA TAA TTT ATA GAC AGA TAA TTT    1006
1007  ATA GAC AGG TAA ATG TGA GTT AAA TAT AAC TCA CAT CCC ACT CAA GAC    1054
1055  ACA AAA CAT TTT CTT AAT CCT AGT ACA TTT TTT TCT GTC CCT TCC CAA    1102
1103  TCA GTG TCC TTT TCT GTT CCA CCC CTA CCA AAA GCA AGT AGT GGT TTG    1150
1151  GTT TCT ATC ATA TAG ATT AAT TTT ACC TGC TCA TAT GAA GGG AAT TGT    1198
1199  ACA TCA TGC ATT CTT TTC TGT TTG CCT TTT TTA AAT TCA GCA TCA TGT    1246
1247  TTT TGT GAT ACA TCC ACA TTG TTG CAT GCA GCT GTA GTT TGT TTC TTT    1294
1295  TTA TTA CCA AGT ACT ATT TCA TTG TAT GAA TAT ATC ACA GTT TAT CCA    1342
1343  TTC TAC TAT TAA GAC AAT TGA GCT ATT TCT AAT TTT CGG CTG CTA TGA    1390
1391  ATA AAG CTG CTA CAA ATA TTT TTG TAC AAG ACT TTT TGT AAA CAT AGG    1438
1439  AGT CCC TTT ATT TTA AAT AAA TAA CTA GTC ATA TCA TTA GGT CCA GTA    1486
1487  ATT GTT GAC AGG CAG GAA CGG GGG ACC ATT GCA TTG TGC CCA AGT AAT    1534
1535  AAT AAA ACT ATT TCA GAT GTA TTA TAT GAT TGA GCA AAT GAG AAA ACA    1582
1583  TGT TGA TGT TGA TGG GAG TCA GGA TGT TCA CTA TGG AAA AAC AAA TAT    1630
1631  ACA AAT ATG AAA TGA GGG AAG GCA AGA AAG AAC CAT GTG GAA ATG GAA    1678
1679  TAG AAT TGG TAT AAA TTC ATA ATT TCT AAA CCA TGT ATA TGT ACG TTT    1726
1727  ATA TGT ATT ATA ATT GCA TAC ACA TGC CTC CAT GCA TAT ATG TGT GTG    1774
1775  ATA ATA CAC ATG CAT TTA TGT GCG TGT GTG TAT ACA CAT GCA TAT ATT    1822
1823  TAC TAA TCC TAT CTG CCA AAA TGG CTT AGA CAC AAA AAC ACC TCA GCA    1870
1871  GAA ATG AAT ATA CCT AGC ACT CAG ATC TTC GTG TCT AAT ATA GTT TGC    1918
1919  CAC TAA AAG GAA CCA AGG CTA CTT GGA AAA ATG GAT GAT TCC AAA GCA    1966
1967  AGG GCA AGG TAG GAA CAA GAT GAG CTT GAA ATA TCT TGT TAT GCC AGA    2014
2015  AAG TAA TGT TAA AAA AAA ATA GAG GTA TAT TGC CAA AAC ATA GAG      2062
2063  CCA GCT TGA AGG GGC TCC CAC TGG CCA AAC TTG AGC CAA TCT GAG AGC    2110
2111  AAA ATA ATT GAG AAA AAT AAA TAA CAA GAT AAT TGA AAA A             2150
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Fig.1.
Nucleotide and deduced amino acid sequences for human P2 protein. Polyadenylation signals are underlined.

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HUMAN   SNKFLGTWKLVSSENFDDYMKALGVGLATRKLGNLAKPTVIISKKGDIITIRTESTFKNT   60
RABBIT  ........................................N.....................
MOUSE   ..............H.............N..................Y.......A....

HUMAN   EISFKLGQEFEETTADNRKTKSIVTLQRGSLNQVQRWDGKETTIKRKLVNGKMVAECKMK  120
RABBIT  ......................I..E..A.....K.............D....V.....
MOUSE   ..........D........A......E....K...K......A.R.T.LD.R..V..I..

HUMAN   GVVCTRIYEKV                                                  131
RABBIT  ...........
MOUSE   ...........
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Fig.2.
Comparison of the deduced amino acid sequence of the P2 proteins from human, rabbit and mouse.

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