We have cloned and characterized the human gene for the 21-kDa brain form of prostaglandin D₂ synthase. The gene was isolated from a human genomic λ library and spans 3600 base pairs. It consists of seven exons and six introns. Southern blot analysis indicates that there is a single copy of the gene in the haploid genome. The transcriptional start site was mapped to a G residue 74 base pairs 5' of the ATG initiation codon. A TATA box-like element (ATATAATA) is situated 21 base pairs upstream of the mRNA start site. The gene was mapped to chromosome 9 bands q34.2-q34.3. The gene bears close resemblance to the genes for murine major urinary protein and ovine β-lactoglobulin.

Prostaglandins are arachidonic acid metabolites that have a wide variety of biological functions (1, 2). Prostaglandin D₂ (PGD₂) is a potent inhibitor of platelet aggregation in vitro and is involved in smooth muscle contraction/relaxation (3). PGD₂ has been implicated in a variety of CNS functions including synaptic transmission (4), homeostatic control of temperature (5), recovery from seizures (6, 7), and release of luteinizing hormone (8). One of the more intriguing aspects of the postulated role of PGD₂ in CNS function is the part it plays in sleep. PGD₂ is involved in sleep initiation and is capable of inducing sleep in rodents and cats (9). The connection between PGD₂ and sleep is further strengthened with the observation that CNS levels of PGD₂ are significantly elevated in patients in advanced stages of sleeping sickness (10). In a series of elegant experiments, Hayashi and co-workers (11-13) have produced a significant body of evidence linking PGD₂ to sleep induction in rats and monkeys.

Prostaglandin H₂ is the highly reactive precursor to PGD₂ with a half-life in solution of approximately 30 min at physiologic pH. It decomposes to a mixture of primarily PGD₂ and PGE₂ (14). This lability has made identification of specific PGD₂ synthases difficult. Several proteins have been shown to accelerate this reaction, including serum albumin, members of the glutathione-S-transferase family of enzymes (15, 16), and an 85-kDa cytosolic protein isolated from rat brain by Shimizu et al. (17). Hayashi and co-workers (18, 19) have identified a brain-specific prostaglandin D₂ synthase (PDS), a 199-amino-acid protein that is homologous to members of the lipocalin superfamily. In an immunohistochemical study, Urade et al. (20) demonstrated that staining with anti-PDS antibodies is primarily neuronal in the brain of 1-2-week-old rats, whereas in the adult rat most of the staining is observed in oligodendrocytes. The appearance of this protein in the maturing oligodendrocyte may therefore be an important marker for the terminal differentiation of the oligodendrocyte. Our interest in this protein arose from our attempts to understand events and proteins involved in oligodendrocytic development. Here, we report on the isolation and characterization of the human gene for PDS and its close structural resemblance to the genes for murine major urinary protein (MUP) and ovine β-lactoglobulin (OVBLG). We have also localized the gene to the long arm of chromosome 9 bands q34.2-q34.3, which is within the region to which subsets of mutations in both tuberous sclerosis and familial torsion dystonia have been mapped (21-24).

MATERIALS AND METHODS

Isolation of the PDS cDNA and Probe Generation—The PDS cDNAs were cloned from a human fetal spinal cord expression library by virtue of the protein's reactivity with a polyclonal antisera raised against the oligodendrocyte-myelin glycoprotein (OMgp) (25). A λgt11 cDNA library derived from spinal cord of a 1-day-old child (American Type Culture Collection) was probed with a polyclonal rat anti-serum raised against OMgp (26). Four positive cDNA clones were isolated from agarose gels using DE81 ion-exchange paper (Whatman). The probe was generated by incorporation of [γ-³²P]CTP through random priming (27) of the cDNA insert using an oligo(dT)₁₄-₁₈ (Pharmacia) spin column. After characterizing and sequencing the overlapping cDNA clones, we discovered that they encode a protein distinct from OMgp. One of the cDNA subclones, B1C, was chosen as a template for probe synthesis because a small truncation at the 3' end had resulted in the loss of the lengthy polyadenylate sequence found on the other clones. The cDNA insert was excised at the flanking EcoRI sites and was isolated from agarose gels using DE81 ion-exchange gel (Whatman). The probe was generated by incorporation of [γ-³²P]CTP through random priming (27) of the cDNA insert using an oligo(dT)₁₄-₁₈ (Pharmacia) spin column. After characterizing and sequencing the overlapping cDNA clones, we discovered that they encode a protein distinct from OMgp. One of the cDNA subclones, B1C, was chosen as a template for probe synthesis because a small truncation at the 3' end had resulted in the loss of the lengthy polyadenylate sequence found on the other clones. The cDNA insert was excised at the flanking EcoRI sites and was isolated from agarose gels using DE81 ion-exchange gel (Whatman). The probe was generated by incorporation of [γ-³²P]CTP through random priming (27) of the cDNA insert using an oligo(dT)₁₄-₁₈ (Pharmacia) spin column. After characterizing and sequencing the overlapping cDNA clones, we discovered that they encode a protein distinct from OMgp. One of the cDNA subclones, B1C, was chosen as a template for probe synthesis because a small truncation at the 3' end had resulted in the loss of the lengthy polyadenylate sequence found on the other clones.
constructed from human genomic DNA isolated from peripheral blood lymphocytes of a single individual. The genomic DNA was partially digested with MboI prior to ligation into the Lambda Fix II vector system (Stratagene) and packaging using the Gigapack II (Stratagene) packaging mix. P2PLK bacteria infected with the λ library were plated, and plaque lifts were obtained using Colony/Plaque Screen (Du Pont) membranes. The library was screened using the probe described above, and six positive clones were plaque purified. After an initial restriction enzyme analysis, three of the six inserts were excised using XbaI and subcloned into pHBluescript KS.

Sequencing of the Genomic Clones—An oligonucleotide-directed approach was used to determine the exon/intron boundaries. Oligonucleotides were designed according to the sequence of the PDS cDNA clone, B1C, and synthesized on an Applied Biosystems 391A automated synthesizer. Sequencing was performed on single- or double-stranded DNA using the dideoxy method of chain termination (18) and Sequenase polymerase (U. S. Biochemicals). Regions of intense secondary structure were resolved using 8% polyacrylamide gels that contained 40% formamide and 7 M urea. Exon/intron boundaries were determined by sequence comparisons between the genomic and BIC cDNA sequences. Sequence analysis was performed using the Seeg and Bestfit programs of the University of Wisconsin Genetics Computer Group Sequence Analysis Software genetics program (29). Intron sizes were determined by direct sequencing, restriction analysis, and/or polymerase chain reaction analysis.

Southern Blot Analysis—7 µg of DNA was digested to completion with restriction endonuclease and electrophoresed in a 0.7% agarose gel. The DNA was capillary blotted onto GeneScreenPlus membranes (Du Pont) and prehybridized and hybridized at 55 °C following the manufacturer's instructions. The probes were synthesized as described above. After an overnight hybridization the membrane was washed twice in 1% SDS, 0.1 × SSC at 65 °C for 30 min and was exposed to x-ray film for 2 days at −70 °C with the use of intensifying screens.

Northern Blot Analysis—Total RNA was isolated from human brain using the guanidinium thiocyanate method of Chomczynski and Sacchi (30). 10 µg of total RNA was electrophoresed through 1% agarose gels containing 3% formaldehyde. The RNA was capillary blotted onto a Gene Screen nylon membrane (Du Pont) and UV cross-linked to the membrane. Prehybridization and hybridization were performed at 42 °C in a 50% formamide buffer. The probe was prepared as described above. After an overnight hybridization the blot was washed following the same procedure as for the Southern blots. The membrane was exposed to x-ray film for 4 days at −70 °C with the use of intensifying screens.

Primer Extension Analysis—Total RNA, isolated as described above, was used as the extension templates. Two oligonucleotide primers, PDS34: CGACGCTCCTCTTACCGATGAGT (+64 to +85) and PDS17: GTGTGCGACACCTACCCTGAC (+87 to +107) were end-labeled by incubation with γ-32P-ATP (7000 Ci/mmol, ICN) and polynucleotide kinase (Boehringer Mannheim) at 37 °C for 45 min. Unincorporated [γ-32P]ATP was removed with a G-25 spin column following precipitation of the labeled primer. The primer had a specific activity of 3 × 108 cpm/µg. 35 µg of total RNA or 50 µg of yeast tRNA control were coprecipitated with 3 × 106 cpm of the primer. The pellet was resuspended in 20 µl of hybridization buffer (50 mM NaCl, 60 mM Tris-Cl, pH 8.4 and 6 mM MgCl2), denatured at 80 °C for 4 min, and slowly cooled to 50 °C. Hybridization was continued for an additional 2 h after which the reaction volume was increased to 50 µl and contained 16 mM dithiothreitol, 400 µM dNTPs and 20 units of RNAsin (Promega) in hybridization buffer. 8 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) were added and the samples were incubated at 42 °C for 90 min. The products were treated with RNase A (Sigma) at room temperature and recovered by ethanol precipitation. Samples were resuspended in 6 µl of TE (Tris-EDTA) and 9 µl of formamide loading buffer and were denatured at 96 °C for 3 min before loading. Single-stranded DNA derived from a genomic subclone (G4CS86) containing the 5' end of the gene, and flanking region was sequenced by standard methods with the primers used in the extension reactions. The extension products and the corresponding sequencing reactions were electrophoresed side by side on a sequencing gel.

Sequence of the PGD2 Synthase Gene—Human genome DNA from a single individual was used to produce Southern blots. When probed with the PDS cDNA probe (Fig. 2), all hybridizing bands were consistent with the size expectation for the PDS mRNA (0.9 kb) (19).

Human genomic DNA from a single individual was used to produce Southern blots. When probed with the PDS cDNA probe (Fig. 2), all hybridizing bands were consistent with the size expectation for the PDS mRNA (0.9 kb) (19).

Sequence of the PGD2 Synthase Gene—The nucleotide sequence of the PDS gene is shown in Fig. 3. The first exon is split between coding and noncoding (74 bp) sequences. The sixth exon is quite small (23 bp) and ends precisely at the stop codon. The seventh and final exon is 163 bp and is entirely noncoding. A polyadenylation signal (AA-TAAA) is found 18 bp upstream from the polyadenylation

![Fig. 1. Structure of the PDS gene. The boxes represent exons and the lines introns. Numbers below the boxes are exon sizes in bp. The shaded portion of the boxes represents the coding portion of the exons. Numbers above the lines represent the intron sizes in bp. Roman numerals refer to the exon number. The location of several restriction sites are shown below.](image-url)
product that maps to the same G residue 74 bp upstream of the initiating ATG was observed when the extension was performed using the 22-base primer PDS34 that covers coding as well as noncoding regions (bases +64 to +85) (Fig. 4). These observations are consistent only with the sequence of the genomic clones.

**Chromosomal Localization of the PGS2 Synthase Gene**—To determine the chromosomal localization of the PDS gene, we performed fluorescence in situ hybridization of a biotin-labeled PDS genomic probe to normal human metaphase chromosomes. Fluorescent signals from biotin-labeled probes are visualized as discrete green-yellow dots on unstained chromosomes; specific signal is frequently observed on all four chromatids. Hybridization of the genomic PDS probe resulted in specific labeling only of chromosome 9 (Fig. 5). Specific labeling of 9q34.2-q34.3 was observed on three (five cells) or all four (20 cells) chromatids of the chromosome 9 homologues in 25 cells examined. Similar results were obtained in three additional hybridization experiments using these probes. Thus, the PDS gene is localized to chromosome 9, bands q34.2-q34.3.

To determine the localization of the PGS2 synthase gene relative to the ABL oncogene (9q34.2) (35), the HXB gene (9q33-q34) (36), and the centromere, we used dual-color fluorescence in situ hybridization. Biotin-labeled PDS and HXB probes were cohybridized with a digoxigenin-labeled ABL probe. The HXB probe hybridized to 9q33, whereas the signals for the ABL and the PDS probes were observed at 9q34.2-q34.3. The chromosome 9 centromere was identified with a probe for pericentromeric β-satellite sequences. The ABL signal (detected with rhodamine) was centromeric to the PDS signal, indicating that the order of these genes is cen-HXB-ABL-PDS-tel.

**Relationship between the PGS2 Synthase Gene and the Genes for Mouse Major Urinary Protein and Ovine β-Lactoglobulin**—The deduced amino acid sequence of PDS places it in a superfamily of proteins that Pervaiz and Brew (37) have termed the lipocalins. The lipocalins are small secretory proteins usually involved in the transport of hydrophobic ligands. We compared the structure of the PDS gene to other members of the lipocalin family whose gene structures are known. Two members of this superfamily have gene structures very similar to that of PDS: mouse major urinary protein (MUP) (38) and ovine β-lactoglobulin (OVBLG) (39). A diagrammatic comparison of the structures of the three genes is shown in Fig. 6. Among the common features are: 1) a first exon that is split between coding and noncoding sequences. 2) The sizes of the second through fifth exons in the PDS gene closely resemble the sizes of these exons in the MUP and OVBLG genes. Their sizes vary among these three genes by five or fewer bp. 3) The sixth exon of each is quite small (23–46 bp) and contains the stop codon. 4) The seventh exon is in general the largest and is entirely noncoding. The coding region of the PDS gene has a 42% identity to the MUP coding sequence and a 44% identity to the OVBLG-coding sequence. A comparison of the 3′-noncoding regions of the PDS gene to the MUP and the OVBLG genes reveals a 42 and 45% identity, respectively. The 5′-untranslated region of the PDS gene is 37% identical to MUP and 50% identical to the OVBLG 5′-untranslated regions.

The rat congener of the MUP gene family are the α2g globulin genes. When the PDS gene sequences are compared to the genomic sequences for rat α2g globulin (40, 41) we find a 42% identity both for the coding and 3′-noncoding portions and a 32% identity for the 5′-untranslated part of the genes.

The intron phasing, i.e. the position within the codon at
FIG. 3. Nucleotide sequence of the human PDS gene. The nucleotide sequence of the exons and flanking intronic regions are presented. Capitalized sequences are present in the cDNA. Sequences that are underlined are noncoding in the cDNA. The deduced amino acid sequence is above the coding sequence. The boxed sequence is the TATA box-like element and the polyadenylation signal is double underlined. The upper of the two numbers refers to the amino acid residue number, the lower refers to the nucleotide number in the cDNA. The numbers within the sequence refer to the approximate size of the intronic sequences not included in the figure.
which the intron interrupts the exon, is conserved in the genes of the lipocalin family (39). The PDS gene possesses this same conserved intron phasing (Fig. 6) whereby the first and sixth intron interrupt between codons, the third through fifth introns interrupt between the first and second base of the codon, and the second intron interrupts between the second and third base of the codon.

When the deduced amino acid sequences of PDS, MUP, and OVPBLG are aligned to maximize their sequence similarities (Fig. 7), we find that the location of the introns within the primary structure of the proteins is conserved as well. PDS contains the conserved amino acids and spacings that are a hallmark of the lipocalins. PDS shares 24% identity and 46% similarity (accounting for conservative substitutions) with MUP and 23% identity and 49% similarity with OVPBLG. A comparison of the deduced amino acid sequences of PDS to \( \alpha_1 \) globulin shows that they are 28% identical and 48% similar. In Fig. 7, we have also aligned the PGD \(_2\) synthase sequence with that of the human retinol-binding protein which is one of the best characterized members of the superfamily and whose tertiary structure has been crystallographically solved (42).

**DISCUSSION**

In this study we report the structure of the PDS gene. The gene is small (3.6 kb) and is composed of seven exons and six introns. We mapped the transcriptional start site to a G residue 74 bp upstream of the ATG initiation codon and located a TATA box like element (ATAATA) 21 bp 5' from the transcriptional start site. Given the structural integrity of the gene and its high sequence similarity to the cDNA for PDS we have concluded that this is the gene for PDS. Southern blot analysis indicates that there is a single copy of the PDS gene in the haploid genome. Northern blot analysis detected only one message in total RNA from adult human brain that hybridizes to the PDS cDNA.

A comparison of the exons of the PDS gene to the previously published cDNA sequence (19) as well as to our cDNA clone

**Fig. 6. Comparison of the structure of the PDS gene to the MUP and OVPBLG genes.** The boxes represent exons with the shaded regions representing the coding portions of the gene. Numbers below the boxes are exon sizes in bp. The lines connecting the boxes represent the introns. The position within the codon at which the intron interrupts is given above the line where 3:1 indicates an intron that interrupts between adjacent codons, 2:3 an interruption between the second and third base of a codon, and 1:2 indicates an intron that interrupts between the first and second base of a codon.

![Diagram](image_url)
reveals numerous differences. The GC content of the PDS message is relatively high at 64% and presented several areas of secondary structure that proved difficult to sequence. It was in these areas that the majority of the sequence discrepancies arose. We had the benefit of comparing our cDNA to that of Nagata et al. (19) as well as to the genomic sequences. Therefore, we are certain of the exonic sequences presented here. Northern blot analysis demonstrates that B1C hybridizes to a single message in human CNS that is the same size as the message detected with the cDNA reported by Nagata et al. (19). Furthermore, under these same conditions the human B1C probe also hybridizes to one message in rat CNS (data not shown) of the same size as the message reported by Urade et al. (18). This occurs in spite of the fact that rat and human sequences are only 71% identical. Primer extension using primers from two different positions on the 5’ end generate only one product. The lack of heterogeneity in the primer extension product argues against the existence of either multiple forms of the PDS message or of other messages very similar to the PDS message. Thus, it is clear that the exonic sequences reported here and the cDNA sequences reported by Nagata et al. (19) are the same.

The presence in and function of PGD₂ in the CNS has been or is rapidly being established for many higher mammals. Yet little is known regarding the presence of PGD₂ or its function in the human CNS. Early studies failed to detect the presence of PGD₂ in cerebrospinal fluid or brain tissue (43) while recent analysis has clearly demonstrated its presence in normal human CSF (10). The presence of the enzyme in the human CNS argues for a functional role for PGD₂ in the CNS. Our data indicate that the message for PDS is indeed abundant in the human brain. While there may be at least one other enzyme possessing the same function as PDS in the brain (17), PDS is the first to have been characterized extensively at the molecular level.

The PDS gene is on chromosome 9 bands q34.2-q34.3 and is telomeric to HXB and ABL. Several of the genes that have been mapped to this region are gelsolin (GSN, q34), δ-aminolevulinic dehydratase (ALAD, q34), argininosuccinate synthetase (ASS, q34-qter), adenylate kinase (AKI, q34.1-q34.2), and the ABO blood groups (ABO, q34.1-q34.2) (44). The observation that the ABO genes and the PDS gene share the same region of chromosome 9 is of interest because it has been found that in 40% of families with tuberous sclerosis the disease locus (TS1) is linked to the ABO blood groups on 9q34 (21, 22). Tuberous sclerosis is an autosomal dominant disease that in the CNS is characterized by tumors and malaligned or disorganized tissue (45).

Familial torsion dystonia is an autosomal dominant disease that has been localized by genetic linkage analysis in two large kindreds to chromosome 9 q32-q34 (23, 24). The disease is characterized, among other things, by involuntary muscle contractions resulting in abnormal postures and movements (46). In one of the kindreds, recent evidence places the gene for torsion dystonia to an 11 centimorgan region between the gelsolin (24) and argininosuccinate synthase genes with the gene most likely located between the ABL oncogene and the argininosuccinate synthase gene. The PDS gene is located in this region and is therefore, based on its position, a candidate gene for torsion dystonia. The identification of polymorphisms distal and around the PDS gene would allow for genetic mapping of the PDS gene in relationship to the tuberous sclerosis and torsion dystonia loci.

The PDS gene has a structure that is almost identical to the MUP and OVLBLG genes. When the coding regions of the PDS gene were compared to coding regions of the MUP and OVLBLG genes they were 42 and 44% identical, respectively. A comparison of each exon of the PDS gene to the corresponding exon in the MUP and OVLBLG genes reveals that no particular exon is more highly or consistently conserved than the other six. When the PDS primary structure is compared to the primary structures of MUP and OVLBLG they are 46 and 45% similar, respectively. When a similar comparison is made to rat α₂b globulin and bovine β-lactoglobulin the similarity rises to 48 and 57%, respectively. The 3’-noncoding regions of the genes were compared and found to have the same level of identity as the coding regions. The high level of conservation found in the 3’-noncoding regions may imply a role for these sequences in mRNA stability or translation efficiency.

The lipocalins can be characterized as small, soluble, secretory proteins that bind small hydrophobic molecules (37). PDS fits well into this class since its substrate, PGH₂, is also a small hydrophobic molecule. It differs from other members of the superfamily in that it has an intracellular localization (47) and that it has enzymatic activity. There are other members of the lipocalin superfamily that have unique localizations. Avian purpurin (48), a protein with which PDS shares 49% similarity, has been found associated with the extracellular matrix and has been implicated in the promotion of cell-substrate adhesion in vitro, yet purpurin is still considered to be a retinol transporter. It is possible that PDS may also possess a functional duality in that it catalyzes the conversion of PGH₂ to PDG₂, but may also transport the product to a second site of action.

The brain-specific expression of the PDS gene and the developmentally associated shift in its expression from neurons to oligodendrocytes may indicate that the PDS gene is controlled in a tissue-specific and developmentally coordinated manner. The knowledge of the regulatory elements of the lipocalin genes is limited. Best studied are the regulatory elements of the o₁b globulin gene, where glucocorticoid response elements have been identified in the upstream region of the gene (49). Furthermore, an in vitro footprinting study has found binding of tissue-specific nuclear factors to the third intron of the o₁b globulin gene (50). It is of interest that the sixth intron of the PDS gene shares some of the sequence elements with the third intron of the o₁b globulin gene. The cloning and characterization of the PDS gene will allow analysis of the transcriptional regulation of the PDS gene in the brain.

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