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Human brain prostaglandin D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins

(prostaglandin D_2/cDNA/lipocalin superfamily/α_1-microglobulin/molecular evolution)

AKIHISA NAGATA*, YASUHIKO SUZUKI*, MAKOTO IGARASHI*, NAOMI EGUCHI*†, HIROYUKI TOH‡, YOSHIHIRO URADE§¶, AND OSAMU HAYAISHI§‖

*Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan; ‡Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, Japan; and §Department of Enzymes and Metabolism, Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565, Japan

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ABSTRACT **cDNAs for glutathione-independent prostaglandin D synthase were isolated from cDNA libraries of human brain. The longest cDNA insert was 837 base pairs long and contained a coding region of 570 base pairs corresponding to 190 amino acid residues with a calculated M_r of 21,016. Between two cDNA inserts isolated from the two different libraries, nucleotide substitutions were observed at 16 positions, including conservative amino acid substitutions at 2 positions and nonconservative substitutions at 5 positions, indicating genetic heterogeneity of this enzyme in humans. The computer-assisted homology search revealed that the enzyme is a member of the lipocalin superfamily, comprising secretory hydrophobic molecule transporters, showing the greatest homology (28.8–29.4% identity; 51.3–53.1% similarity) to α_1-microglobulin among the members of this superfamily. In a phylogenetic tree of the superfamily, this enzyme, α_1-microglobulin, and the γ chain of the complement component C8 form a cluster separate from the other 14 members. The two distinctive characteristics of glutathione-independent prostaglandin D synthase, as compared to the other members of this superfamily, are its enzymatic properties and its association with membranes that were probably acquired after evolutionary divergence of the two lipocalins. Based on the observed sequence homology, the tertiary structure of the enzyme was deduced to consist of an eight-stranded anti-parallel β-barrel forming a hydrophobic pocket. Furthermore, the Cys-65 residue in the pocket, which is conserved only in the human and rat enzymes but not in other lipocalins, was considered to be a putative active site of the enzyme.**

Prostaglandin (PG) D_2 is a major PG in rat brain and shows several central actions *in vivo* such as sleep induction, regulation of body temperature, anticonvulsion, suppression of luteinizing hormone release, nociception, and modulation of odor responses. *In vitro* it also regulates the growth and differentiation of rat glioma C6BU-1 cells and induces Ca^{2+} influx and cGMP formation in mouse neuroblastoma–rat glioma hybrid NG108-15 cells (for reviews, see refs. 1 and 2). In brief, PGD_2 functions as a neuromodulator and/or trophic factor in the central nervous system.

Glutathione (GSH)-independent PGD synthase [prostaglandin-H_2 D-isomerase; (5*Z*,13*E*)-(15*S*)-9α,11α-epidioxy-15-hydroxyprosta-5,13-dienoate D-isomerase, EC 5.3.99.2] catalyzes the conversion of PGH_2 to PGD_2 in the presence of various sulfhydryl compounds (3). This enzyme is responsible for biosynthesis of PGD_2 in the brain, while several other enzymes, such as GSH-requiring PGD synthase and GSH *S*-transferase, also catalyze the reaction to produce PGD_2 and are present in various other rat tissues (4). The enzyme is localized in the central nervous system (4) and associated tissues, such as the retina (5) and cochlea (6). Furthermore, postnatal changes in the cellular localization of this enzyme have been observed in the brain (7) and cochlea (6). For example, the enzyme is distributed in most neurons in the brain of 1- to 2-week-old rats, but the major localization site shifts to oligodendrocytes in adult animals (7). Therefore, the enzyme is likely to play important roles in both maturation and maintenance of the central nervous system.

There is also a significant species difference in profiles of PGs in the brain (8). Previously, PGD_2 had not been thought to be produced in human brain (8, 9). However, a considerable amount of PGD_2 was detected in postmortem human brains by both radioimmunoassay and gas chromatography/mass spectrometry (10). Furthermore, PGD_2 was found to be produced enzymatically in the human brain homogenate (11), yet little information is available for PGD synthase in human brain.

Here we report on the isolation of cDNAs encoding GSH-independent PGD synthase in human brain and show the nucleotide and deduced amino acid sequences of this enzyme.** By homology searching in a data base of protein primary structure, the enzyme is shown to be a member of the lipocalin superfamily consisting of hydrophobic molecule transporters (12). The tertiary structure of the enzyme is deduced by analogy with those of retinol-binding protein (13, 14), β-lactoglobulin (15, 16), and insecticyanin (17, 18), all of which belong to this superfamily.

MATERIALS AND METHODS

Screening of Phage Clones Containing the cDNA for GSH-Independent PGD Synthase in Human Brain. A human brain cDNA library within a λgt11 vector (Clontech) was immunoscreened with a polyclonal antibody against PGD synthase purified from rat brain (3). Another cDNA library was constructed from human brain mRNA (Clontech) by the method of Gubler and Hoffman (19) in which the size-selected double-stranded cDNA [0.6–2.0 kilobases (kb)] was ligated into the *Eco*RI site of the vector λgt10. This library was screened with a ^{32}P-labeled oligonucleotide insert obtained from the λgt11 library.

Northern and Southern Blotting. Total RNA was extracted from rat brain by the method of Chomczynski and Sacchi (20) and the mRNA was purified by oligo(dT)-cellulose column

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Abbreviations: PG, prostaglandin; GSH, glutathione.

†Present address: Department of Enzymes and Metabolism, Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565, Japan.
¶Present address: International Research Laboratories, CIBA-GEIGY (Japan) Limited, 10-66 Miyuki-cho, Takarazuka 665, Japan.
‖To whom reprint requests should be addressed.

**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M61900, M61901, and M61902).

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CTCCTCCTGCACACCTTCCGCACACCTCCCTCGCTCTCCCACACCACTGGCACCAGGCCCCGCACACCTGCTCGGCTGCAGGAGAATGGCTACTCATCACACGCTGTGGATGGGACTGGT  120
                                                                                                  M  A  T  H  H  T  L  W  M  G  L  V
CCTGCTGGGGCTGCTGGGCGGCCTACAGGCAGCACCCGAGGCCCAGGTCTCCGTGCAGCCCAACTTCCAGCCGGACAAGTTCCTGGGGCGCTGGTTCAGCGCGGGCCTCGCCTCCAACTC  240
                                                  .....A.....
  L  L  G  L  L  G  G  L  Q  A  A  P  E  A  Q  V  S  V  Q  P  N  F  Q  P  D  K  F  L  G  R  W  F  S  A  G  L  A  S  N  S
                                                           (Q)
GAGCTGGCTCCAGGAGAAGAAGGCAGCGCTGTCCATGTGCAAGTCGGTGGTGGCCCCTGCGGCGGATGGTGGCTTCAACCTGACCTCCACCTTCCTCAGGAAAAACCAGTGTGAGACCCG  360
.....G.....A.....C.....
  S  W  L  Q  E  K  K  A  A  L  S  M  C  K  S  V  V  A  P  A  A  D  G  G  F  N  L  T  S  T  F  L  R  K  N  Q  C  E  T  R
     (R)                                                    (T)     (L)
AACCATGCTGCTGCAGCCCGGGGACTCCCTCGGCTCCTACAGCTACCGGAGTCCCCACTGGGGCAGCACCTACTCTGTGTCAGTGGTGGAGACTGACTACGACCACTACGCCCTGCTGTA  480
.....C..G.....G.....G.....
  T  M  L  L  Q  P  G  D  S  L  G  S  Y  S  Y  R  S  P  H  W  G  S  T  Y  S  V  S  V  V  E  T  D  Y  D  H  Y  A  L  L  Y
        (A) (G)                                                                                    (Q)
CAGCCAGGGCAGCAAGGGCCCCGGCGAGGACTTCCGCATGGCCACCCTCTACAGCCGAACCCAGACCCCCAGGGCTGAGTTAAAGGAGAAATTTACCGCCTTCTGCAAGGCCCAGGGCTT  600
.....
  S  Q  G  S  K  G  P  G  E  D  F  R  M  A  T  L  Y  S  R  T  Q  T  P  R  A  E  L  K  E  K  F  T  A  F  C  K  A  Q  G  F
CACAGAGGATTCCATTGTCTTCCTGCCCCAAACCGATAAGTGCATGACGGAACAATAGGACTCCCCAGAGCTGAAGCTGGGACCGCAGCCAGCCAGGTGACCCCTGCGATCTGGATGTTT  720
.....G.....T.C.G.....CA.CG
  T  E  D  S  I  V  F  L  P  Q  T  D  K  C  M  T  E  Q  *
CCGCTCTGTTCCTTCCCCGAGCCCCTGCCCCGGCTCCCCGCCAAAGCACCCCTGCCCCCTCGGGCTTCCTCCTGGCTCTGCGGAATAAACTCCGGAAGCAAGTCTGTAAAAAAAAAA  837
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FIG. 1. Nucleotide and deduced amino acid sequences of GSH-independent PGD synthase in human brain. The sequences obtained from the λHBDS-1 insert (lower sequences) are numbered and aligned below those from the λHBDS-2 insert (upper sequences). Dots in the HBDS-1 sequence indicate nucleotide residues identical between these two cDNA inserts. The substituted amino acid residues in the HBDS-1 sequence are shown in parentheses. Asterisk, stop codon. A polyadenylylation signal is underlined.

chromatography. The mRNAs of rat and human brains were denatured with 1 M glyoxal in 50% dimethyl sulfoxide, electrophoresed on a 1.5% agarose gel, and transferred to a Biodyne transfer membrane (Pall). Human genomic DNA was extracted from KD cells (American Type Culture Collection). The DNA (5 μg) was digested with various restriction endonucleases, fractionated on a 0.8% agarose gel, and transferred to the membrane. The blots were hybridized with the ^{32}P-labeled oligonucleotide cDNA insert.

DNA Sequencing. The nucleotide sequence was determined by the dideoxynucleotide chain-termination procedure (21) after subcloning into M13mp18 and M13mp19 vectors.

Homology Search, Sequence Alignment, and Construction of Phylogenetic Tree. The deduced amino acid sequence of human brain PGD synthase was subjected to a homology search in a data base of protein primary structure, Protein Research Foundation (release 90.9) (22), with a program for weak homology detection (23). A multiple alignment was constructed from the pairwise alignments obtained with the program and modified by visual inspection to increase similarity. The statistical significance of the sequence similarity was evaluated for each aligned pair by a jumbling test with 100 pairs of randomized sequences (24). MDM_{78} (24) was used as a score matrix for the homology search, the alignment, and the statistical test. A score of −60 was assigned for a gap. A continuous gap was treated as a single substitution. An unrooted phylogenetic tree was constructed by the neighbor-joining method (25) from the differences among the aligned sequences after correction for multiple substitutions at a site (26, 27).

RESULTS AND DISCUSSION

Nucleotide and Deduced Amino Acid Sequences of GSH-Independent PGD Synthase cDNA in Human Brain. Antibody against GSH-independent PGD synthase purified from rat brain (3) showed ≈10% cross-reactivity against the human brain enzyme in immunotitration and dot blot analysis. The antibody was used for immunoscreening of a λgt11 library of human brain cDNA, giving a positive clone (λHBDS-1) [541 base pairs (bp)]. Since the insert did not contain the full-length coding sequence, we screened a λgt10 library prepared from size-selected (0.6–2.0 kb) human brain cDNA by plaque hybridization with the λHBDS-1 insert as a probe and isolated a single clone (λHBDS-2) containing the longest insert of 837 bp.

The long cDNA insert (HBDS-2) contained a 5′-untranslated region of 85 bp, a coding region of 570 bp, and a TAG stop codon followed by 169 bp of 3′-untranslated region ending with a poly(A) tail of 10 bp (Fig. 1). The coding sequence corresponds to 190 amino acid residues, including the first methionine, with a calculated M_r of 21,016. The short insert (HBDS-1) started from nucleotide residue 169 in the coding region and ended at nucleotide residue 709 in the 3′-untranslated region. Between the two inserts, nucleotide substitutions were observed at 16 positions (8 bp in the coding region of 487 bp and 8 bp in the 3′-untranslated region of 51 bp). The substitutions in the coding region resulted in conservative amino acid substitutions at 2 positions (residues 73 and 99 of HBDS-2), nonconservative substitutions at 5 positions (residues 36, 56, 77, 100, and 127), and a silent substitution (residue 129). In a Northern blot of human brain mRNA probed with the ^{32}P-labeled cDNA insert (Fig. 2*A*), a single positive transcript was observed at a position of ≈900

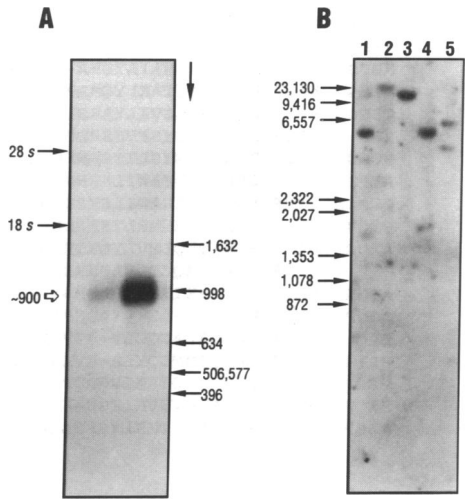

FIG. 2. Northern and Southern blot analyses using the cDNA for GSH-independent PGD synthase in human brain (HBDS-1) as a probe. (*A*) In Northern blots, 1 μg of mRNA from rat brain (left lane) and human brain (right lane) were electrophoresed in a 1.5% agarose gel. (*B*) In Southern blots, 5 μg of human genomic DNA digested with *Bam*HI (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), *Sac* I (lane 4), and *Sma* I (lane 5) were run in a 0.8% agarose gel. The position of the mRNA for PGD synthase is shown by an open arrow in *A*. Horizontal arrows indicate the positions of molecular weight size markers: rRNA (on the left in *A*), the cleavage products of pBR322 DNA cut with *Hin*fI and *Hin*fI/*Eco*RI (on the right in *A*), and those of λ phage DNA cut with *Hin*dIII and of φX174 DNA cut with *Hae* III (on the left in *B*).

FIG. 3. (*Legend appears on the opposite page.*)

bp. In Southern blots using the cDNA probe (Fig. 2*B*), human genomic DNA showed a simple fragmentation profile after endonuclease digestion, suggesting that the two different cDNAs obtained are not derived from gene duplication of the enzyme. Therefore, the polymorphism of the cDNA for human brain PGD synthase indicates the genetic heterogeneity of this enzyme in humans. Such allelic variants have not been detected in nearly inbred rats (28).

The homology of nucleotide sequences between the human and rat enzymes is 75% and 80% in the coding region of HBDS-1 and -2 inserts, respectively, decreasing to 63% in the 5′-untranslated region and <20% in the 3′-untranslated region. The deduced amino acid sequences of the human (HBDS-2) and rat enzymes show 71% identity and 83% similarity, including 26 conservative substitutions, 37 nonconservative substitutions, and a deletion of 1 amino acid in a total of 190 residues (Fig. 3*A*). Among 7 amino acid substitutions between the two human clones, 6 residues of HBDS-1 are identical to those in the rat enzyme and the remaining residue is similar, indicating that HBDS-1 has greater homology with the rat enzyme than HBDS-2. Among the deduced amino acid sequences of the human and rat enzymes, highly conserved domains are observed at 7 positions (residues 10–18, 37–54, 67–76, 78–92, 117–126, 135–153, and 177–187). These regions contained a hydrophobic core of signal sequences (GLVLLGLLG in residues 10–18), two N-glycosylation sites (residues 51–53 and 78–80), and 2 of 3 conserved cysteine residues (residues 89 and 186). In contrast, 6, 8, and 8 of 37 nonconservative substitutions occur within residues 55–61, 100–116, and 154–176, respectively, indicating that these regions are under fewer functional constraints.

As judged by the net charges of protein structures of HBDS-1 (+3), HBDS-2 (±0), and the rat enzyme (−3), the human enzyme appears to be more basic than the rat enzyme (pI 4.35–4.75) (7). This may explain, in part, the difference in elution profiles of the enzyme activity in DEAE-Sepharose column chromatography; i.e., the enzyme from human brain binds weakly to this resin in 10 mM potassium phosphate (pH 7.0) and passes through completely during washing (11), whereas the rat brain enzyme binds tightly to the resin and is eluted only with 0.1 M NaCl under the same conditions (3).

Both the human and rat enzymes have no membrane-spanning domains as judged by the hydrophobicity analysis of the deduced amino acid sequences. This is consistent with the fact that the PGD synthase activity in rat brain (3) and human cerebral cortex (11) is readily dissociated from the membrane and is recovered in the cytosolic fraction without detergents. However, the enzyme is N-glycosylated (28) and has been localized immunocytochemically to rough-surfaced endoplasmic reticulum and the outer nuclear membrane of oligodendrocytes in adult rats (7). Therefore, this enzyme is considered to be a membrane-associated, but not integral, protein facing the luminal and/or extracellular space. This has advantages for effective production of PGD_2 from PGH_2, which is unstable in an aqueous environment, and for rapid release of PGD_2, a member of relatively hydrophilic PGs (48), into extracellular spaces.

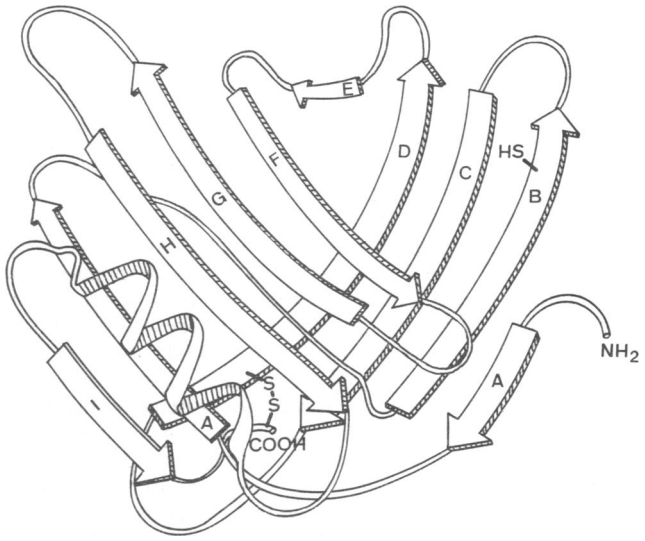

FIG. 4. Schematic representation of the deduced tertiary structure of GSH-independent PGD synthase. Arrows indicate the strands of β-sheets. Strands A–I comprise the residues around the aligned positions of 35–52, 78–83, 91–96, 107–112, 117–127, 132–147, 153–160, 171–173, and 205–213; and the α-helix of 185–199. The Cys-89 and Cys-186 residues (at alignment positions of 105 and 219) are highly conserved among members of the lipocalin family and form a disulfide bond (30).

Homology of GSH-Independent PGD Synthase with Members of the Lipocalin Superfamily. The computer-assisted homology search revealed that the primary structure of this enzyme is homologous with those of members of the lipocalin superfamily (Fig. 3*A*). This superfamily consists of various small (160–190 amino acid residues) secretory proteins sharing a common feature for binding and transport of small hydrophobic molecules (12). GSH-independent PGD synthase also binds to small hydrophobic molecules, such as PGH_2, 1-chloro-2,4-dinitrobenzene, bilirubin, and indocyanine green (Y.U., unpublished data) and shares amino acid residues highly conserved in the lipocalins (30). Among 16 members of the superfamily identified thus far, PGD synthase shows the greatest homology with α_1-microglobulin (28.8–29.4% identity and 51.3–53.1% similarity with a normalized alignment score of 7.1–9.3 SD in a region aligned through the members from positions 43–219), suggesting a close evolutionary relationship between this protein and PGD synthase. PGD synthase, together with α_1-microglobulin and the γ chain of the complement component C8, is part of a cluster distinct from other clusters in a phylogenetic tree of the members of the superfamily (Fig. 3*B*). Two distinctive characteristics of PGD synthase, as an enzyme and as a membrane-associated protein, were probably acquired after the divergence from α_1-microglobulin and the γ chain of the

Fig. 3. (*A*) Alignment of GSH-independent PGD synthase with members of the lipocalin superfamily. The predicted cleavage sites of a putative signal sequence (29) (arrows) and the N-glycosylation sites (open triangles) of the enzyme and the amino acid residues reportedly conserved in the lipocalin superfamily (30) (solid circles) are denoted on the top. Unidentified amino acid residues, positions of the stop codon, and inserted gaps in the alignment are indicated by dots, asterisks, and dashes, respectively. The amino acid residues of exact matches and conservative substitutions against those of HBDS-2 are shown in red and green, respectively. (*B*) Unrooted phylogenetic tree of the lipocalin superfamily. Scale bar represents branch length corresponding to 0.1 amino acid substitution per site. Abbreviations and sources are as follows: HBDS, human brain PGD synthase; RBDS, rat brain PGD synthase (28); A1MG, human α_1-microglobulin (31); C8GC, human γ chain of the complement component C8 (32, 33); RBP, rat retinol-binding protein (34); PURN, chicken purpurin (35); ALPD, human apolipoprotein D (36); INSCN, tobacco hornworm (*Manduca sexta*) insecticyanin (37); P20K, chicken quiescence-specific polypeptide 20K (38, 39); A2UG, rat α_2-urinary globulin (40); OBP, rat odorant-binding protein (41); APHR, hamster aphrodisin (42); A1GP, human α_1-acid glycoprotein (43); BLG, ovine β-lactoglobulin (30); PP14, human placental protein 14 (44); VEGP, rat von Ebner's gland protein (45); BWGP, frog (*Rana pipiens*) Bowman's gland protein (46); ADEP, rat androgen-dependent epididymal secretory 18.5-kDa protein (47).

complement component C8 during evolution of the lipocalin superfamily.

The tertiary structure of a protein is more conserved than the primary structure during evolution. In fact, retinol-binding protein, β-lactoglobulin, and insecticyanin (bilin-binding protein) show only a weak homology with each other (13.3–16.8% identity and 32.9–34.6% similarity) but have remarkably similar tertiary structures with an α-helix and an eight-stranded anti-parallel β-barrel (13–18). PGD synthase shows about the same degree of homology with these three lipocalins (13.9–22.8% identity and 28.9–42.4% similarity). It is therefore likely that PGD synthase also has a three-dimensional structure similar to those of these three lipocalins. Secondary structure prediction of PGD synthase suggests that several regions of the enzyme, corresponding to nine β-strands (A–I) and a three-turn α-helix of the three lipocalins, show high β propensities and an α propensity, respectively. Since the putatively assigned six β-strands (B, C, D, F, G, and H) of PGD synthase are enriched in hydrophobic amino acid residues, these regions might form a hydrophobic pocket as in the three lipocalins (Fig. 4). PGH_2, a substrate for the enzyme, is considered to bind to the hydrophobic pocket like other lipophilic ligands. The Cys-65 residue in the hydrophobic pocket is conserved only in the human and rat enzymes (at an alignment position of 78) but not in other members of the superfamily. Since the enzyme requires sulfhydryl compounds for the reaction and is inactivated by treatments with sulfhydryl modifiers, the cysteine residue may play a key role in the catalytic reaction of PGD synthase.

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1. Hayaishi, O. (1988) *J. Biol. Chem.* **263,** 14593–14596.
2. Ito, S., Narumiya, S. & Hayaishi, O. (1989) *Prostaglandins Leukotrienes Essent. Fatty Acids* **37,** 219–234.
3. Urade, Y., Fujimoto, N. & Hayaishi, O. (1985) *J. Biol. Chem.* **260,** 12410–12415.
4. Ujihara, M., Urade, Y., Eguchi, N., Hayashi, H., Ikai, K. & Hayaishi, O. (1988) *Arch. Biochem. Biophys.* **260,** 521–531.
5. Goh, H., Urade, Y., Fujimoto, N. & Hayaishi, O. (1987) *Biochim. Biophys. Acta* **921,** 302–311.
6. Tachibana, M., Fex, J., Urade, Y. & Hayaishi, O. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7677–7680.
7. Urade, Y., Fujimoto, N., Kaneko, T., Konishi, A., Mizuno, N. & Hayaishi, O. (1987) *J. Biol. Chem.* **262,** 15132–15136.
8. Wolfe, L. S. (1982) *J. Neurochem.* **38,** 1–14.
9. Abdel-Halim, M. S., von Holst, H., Meyerson, B., Sachs, C. & Änggård, E. (1980) *J. Neurochem.* **34,** 1331–1333.
10. Ogorochi, T., Narumiya, S., Mizuno, N., Yamashita, K., Miyazaki, H. & Hayaishi, O. (1984) *J. Neurochem.* **43,** 71–82.
11. Ogorochi, T., Ujihara, M. & Narumiya, S. (1987) *J. Neurochem.* **48,** 900–909.
12. Pervaiz, S. & Brew, K. (1987) *FASEB J.* **1,** 209–214.
13. Newcomer, M. E., Jones, T. A., Åqvist, J., Sundelin, J., Eriksson, U., Rask, L. & Peterson, P. A. (1984) *EMBO J.* **3,** 1451–1454.
14. Cowan, S. W., Newcomer, M. E. & Jones, T. A. (1990) *Proteins* **8,** 44–61.
15. Papiz, M. Z., Sawyer, L., Eliopoulos, E. E., North, A. C. T., Findlay, J. B. C., Sivaprasadarao, R., Jones, T. A., Newcomer, M. E. & Kraulis, P. J. (1986) *Nature (London)* **324,** 383–385.
16. Monaco, H. L., Zanotti, G., Spadon, P., Bolognesi, M., Sawyer, L. & Eliopoulos, E. E. (1987) *J. Mol. Biol.* **197,** 695–706.
17. Huber, R., Schneider, M., Epp, O., Mayr, I., Messerschmidt, A., Pflugrath, J. & Kayser, H. (1987) *J. Mol. Biol.* **195,** 423–434.
18. Holden, H. M., Rypniewski, W. R., Law, J. H. & Rayment, I. (1987) *EMBO J.* **6,** 1565–1570.
19. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
20. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
22. Seto, Y., Ihara, S., Kohtsuki, S., Ooi, T. & Sakakibara, S. (1988) in *Computational Molecular Biology*, ed. Lesk, A. M. (Oxford Univ. Press, New York), pp. 27–37.
23. Nishikawa, K., Nakashima, H., Kanehisa, M. & Ooi, T. (1987) *Protein Sequences Data Anal.* **1,** 107–116.
24. Schwartz, R. M. & Dayhoff, M. O. (1978) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Natl. Biomed. Res. Found., Washington), Vol. 5, Suppl. 3, pp. 353–358.
25. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4,** 406–425.
26. Zuckerkandl, E. & Pauling, L. (1965) in *Evolving Genes and Proteins*, eds. Bryson, V. & Vogel, H. J. (Academic, New York), pp. 97–166.
27. Margoliash, E. & Smith, E. L. (1965) in *Evolving Genes and Proteins*, eds. Bryson, V. & Vogel, H. J. (Academic, New York), pp. 221–242.
28. Urade, Y., Nagata, A., Suzuki, Y., Fujii, Y. & Hayaishi, O. (1989) *J. Biol. Chem.* **264,** 1041–1046.
29. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690.
30. Ali, S. & Clark, J. (1988) *J. Mol. Biol.* **199,** 415–426.
31. Kaumeyer, J. F., Polazzi, J. O. & Kotick, M. P. (1986) *Nucleic Acids Res.* **14,** 7839–7850.
32. Ng, S. C., Rao, A. G., Zack Howard, O. M. & Sodetz, J. M. (1987) *Biochemistry* **26,** 5229–5233.
33. Doolittle, R. F. (1989) in *Prediction of Protein Structure and the Principles of Protein Conformation*, ed. Fasman, G. D. (Plenum, New York), pp. 599–623.
34. Laurent, B. C., Nilsson, M. H. L., Bavik, B. O., Jones, T. A., Sundelin, J. & Peterson, P. A. (1985) *J. Biol. Chem.* **260,** 11476–11480.
35. Schubert, D., LaCorbiere, M. & Esch, F. (1986) *J. Cell Biol.* **102,** 2295–2301.
36. Drayna, D. T., McLean, J. W., Wion, K. L., Trent, J. M., Drabkin, H. A. & Lawen, R. M. (1987) *DNA* **6,** 199–204.
37. Riley, C. T., Barbeau, B. K., Keim, P. S., Kezdy, F. J., Heinrikson, R. L. & Law, J. H. (1984) *J. Biol. Chem.* **259,** 13159–13165.
38. Bedard, P.-A., Yannoni, Y., Simmons, D. L. & Erikson, R. L. (1989) *Mol. Cell. Biol.* **9,** 1371–1375.
39. Cancedda, F. D., Dozin, B., Rossi, F., Molina, F., Cancedda, R., Negri, A. & Ronchi, S. (1990) *J. Biol. Chem.* **265,** 19060–19064.
40. Ichiyoshi, Y., Endo, H. & Yamamoto, M. (1987) *Biochim. Biophys. Acta* **910,** 45–51.
41. Pevsner, J., Reed, R. R., Feinstein, P. G. & Snyder, S. H. (1988) *Science* **241,** 336–339.
42. Henzel, W. J., Rodriguez, H., Singer, A. G., Stults, J. T., Macrides, F., Agosta, W. C. & Niall, H. (1988) *J. Biol. Chem.* **263,** 16682–16687.
43. Dente, L., Ciliberto, G. & Cortese, R. (1985) *Nucleic Acids Res.* **13,** 3941–3952.
44. Julkunen, M., Seppälä, M. & Jänne, O. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8845–8849.
45. Schmale, H., Holtgreve-Grez, H. & Christiansen, H. (1990) *Nature (London)* **343,** 366–369.
46. Lee, K. H., Wells, R. G. & Reed, R. R. (1987) *Science* **235,** 1053–1056.
47. Brooks, D. E., Means, A. R., Wright, E. J., Singh, S. P. & Tiver, K. K. (1986) *J. Biol. Chem.* **261,** 4956–4961.
48. Yokoyama, S., Kimura, T., Nakagaki, M., Hayaishi, O. & Inaba, K. (1986) *Chem. Pharm. Bull.* **34,** 455–462.