Two forms of dipeptidyl peptidase IV (DPP) were purified from rat liver plasma membranes: a membrane form (mDPP) extracted with Triton X-100 and a soluble form (sDPP) prepared by treatment with papain. Apparent molecular masses of mDPP and sDPP were 109 and 105 kDa, respectively, when determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The NH₂-terminal sequences of the two forms were found to be completely different from each other.

For further information on the molecular structure, we constructed a λ gt11 liver cDNA library and isolated two cDNA clones for DPP, λDPP37 and λD5. The 3.5-kilobase cDNA insert of λDPP37 contains an open reading frame that encodes a 787-residue polypeptide with a calculated size of 86,107 Da, which is in reasonable agreement with that of DPP (87 kDa) immunoprecipitated from cell-free translation products. Eight potential N-linked glycosylation sites were found in the molecule, accounting for the difference in mass between the precursor and mature forms. Of particular interest is that the deduced NH₂-terminal sequence with a characteristic signal peptide is completely identical to that determined for mDPP. In addition, the NH₂-terminal sequence of sDPP is identified in the predicted sequence starting at the 35th position from the NH₂ terminus. These results indicate that the signal peptide of DPP is not cleaved off during biosynthesis but functions as the membrane-anchoring domain even in the mature form. It is also found that the primary structure thus predicted has striking homology to that of gp110, a bile canaliculus domain-specific membrane glycoprotein (Hong, W., and Doyle, D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7068-7076).

Primary Structure of Rat Liver Dipeptidyl Peptidase IV Deduced from Its cDNA and Identification of the NH₂-terminal Signal Sequence as the Membrane-anchoring Domain*

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Shigenori Ogata, Yoshio Misumi, and Yukio Ikehara†
From the Department of Biochemistry and the Joint Laboratory for Pathological Biochemistry, Fukuoka University School of Medicine, Jonan-ku, Fukuoka 814-01, Japan

Dipeptidyl peptidase IV (DPP)† (EC 3.4.14.5) is a serine peptidase that cleaves dipeptides from the NH₂-terminal end of peptide chains provided that the penultimate residue is proline (1). The enzyme is an intrinsic membrane glycoprotein, and the highest activity is found in the kidney and the intestinal brush-border membrane (2, 3). Although its activity is relatively low in liver, DPP is also localized in the apical domain, bile canaliculus membrane of hepatocytes (4-6). The purified enzyme is found to be dimeric, comprising two identical subunits of 110-130 kDa (4, 7) which are variable depending on species and tissue, possibly due to the extent of glycosylation (4, 6-9).

Several hydrolases in the kidney and the intestinal brush-border membrane have been studied in detail for their orientation in the membrane (2,3). These enzymes, including DPP, aminopeptidases A and N, neutral endopeptidase (EC 3.4.24.11), γ-glutamyl transpeptidase, and sucrase-isomaltase, have a common feature in their membrane topology. Most of their protein mass, including the catalytic site, protrudes on the extracellular side. Comparisons of the NH₂-terminal sequences of the detergent- and proteinase-released forms suggest that their anchoring domain is located in the NH₂-terminal region (2, 10-12). Recently this mode of anchoring has been confirmed by cloning and sequencing of the cDNAs for γ-glutamyl transpeptidase (13, 14), sucrase-isomaltase (15), and neutral endopeptidase (EC 3.4.24.11) (16) but not yet for DPP.

To study in more detail the membrane anchoring and primary structure of DPP, we have cloned and sequenced its cDNA. The deduced amino acid sequence was compared with the NH₂-terminal sequence of the detergent- and papain-solubilized forms, demonstrating that DPP has an uncleaved signal sequence at its NH₂ terminus functioning as the membrane-anchoring domain.

**EXPERIMENTAL PROCEDURES**

Materials—Fast Garnet GBC salt (o-aminoszotoluene, diazonium salt) was obtained from Sigma. EcoRI linker (pGGAATTCC) and wheat germ lectin-Sepharose 6MB were from Pharmacia LKB Biochemistry Inc.; [α-²⁵P]dCTP (400 or 3000 Ci/mmol), [α-³²P]dCTP (1200 Ci/mmol), and [³⁵S]methionine (1120 Ci/mmol) from Du Pont-New England Nuclear; Xgt.11 from Stratagene (San Diego, CA); the cDNA synthesis kit from Amersham Corp.; the random primer DNA-labeling kit from Nippon Gene (Toyama, Japan); the Sequenase™ DNA-sequencing kit from United States Biochemical Corp. (Cleveland, OH); horseradish peroxidase-conjugated anti-rabbit IgG antibody and Bio-Gel A-50 from Bio-Rad; G-50-p-nitroaniline from Protein Research Foundation (Osaka, Japan). Various DNA-modifying enzymes and restriction endonucleases were obtained from New England Biolabs (Beverly, MA), Nippon Gene, and Takara Shuzo (Kyoto, Japan).

**Purification of Two Forms of DPP**—Plasma membranes were isolated from Donryu rat livers as described previously (17, 18). A soluble form of DPP (sDPP) was purified as follows. Plasma membranes...
were suspended in 20 mM Tris-HCl (pH 7.5) containing 5 mM l-cysteine (protein concentration, about 20 mg/ml). Papain (final concentration, 1.5 mg/ml) was added to the membrane suspension, and the mixture was stirred at 37°C for 3 h followed by centrifugation at 105,000 g for 1 h. The resulting supernatant was subjected to (NH₄)₂SO₄ precipitation. Previous reports have indicated that the partitioning between precipitated and non-precipitated fractions varied with the conditions of precipitation. In the present experiments, (NH₄)₂SO₄ was dissolved in 3 ml of 20 mM Tris-HCl (pH 7.5) containing 0.2 M NaCl and subjected to gel filtration through a Sephacryl S-300 column (2.5 X 100 cm) equilibrated with the above buffer. Fractions with DPP activity were pooled and then applied to a column containing Sepharose 4B coupled with the purified sDPP (20). The resultant library of 1.4 × 10⁹ phages was divided into two portions, 8 × 10⁹ phages, and 2 × 10⁹ phages were pooled and concentrated to a small volume of 20 mM Tris-HCl (pH 7.5) containing 0.5 M NaCl, adsorbed proteins including DPP were eluted with 0.2 M GlcNAc in the same buffer. Fractions with DPP activity were pooled and concentrated to a small volume of 20 mM Tris-HCl (pH 7.5) in an Amicon Corp., ultrafiltration cell with an XM-50 membrane. Aliquots of the sample were subjected to preparative polyacrylamide gel electrophoresis (PAGE) at pH 8.5 followed by staining the gels for enzyme activity (19). Stained areas of the gels were cut out and homogenized in 25 ml of 20 mM Tris-HCl (pH 8.5). The homogenates were stirred at 4°C for 2-3 h and then centrifuged at 30,000 × g for 30 min. Samples obtained by repeating PAGE/extraction were combined and concentrated to about 2 ml. After these purification steps were repeated several times, we finally obtained 3.2 mg of the purified sDPP from 1.079 mg of plasma membranes used.

A membrane form of DPP (mDPP) was purified as follows. The plasma membranes possessing activity were adjusted to contain 0.5% Triton X-100, 0.5% Triton X-100, and 20 mM Tris-HCl (pH 7.5) and stirred at 4°C for 30 min followed by centrifugation at 105,000 × g for 1 h. The resulting supernatant was applied to an Affi-Gel Blue column (3 X 30 cm). When the column was subjected to stepwise elutions with 0.6, 1.0, and 1.5 M NaCl in 20 mM Tris-HCl (pH 7.5) containing 0.2 mM MgCl₂ and 0.5% Triton X-100, most of the DPP activity was eluted with 1.0 M NaCl. Fractions of the activity peak were pooled and subjected successively to wheat germ lectin-Sepharose chromatography and PAGE in the presence of Triton X-100 as described for the sDPP preparation. Finally, 3.1 mg of the purified mDPP was obtained from 916 mg of plasma membranes under these conditions.

Isolation of CNBr Cleavage Peptides—The purified sDPP was cleaved with 2.5 M CNBr in 70% (v/v) formic acid for 24 h at room temperature (20). The sample thus treated was subjected to high performance liquid chromatography (HPLC) through a Superose 12 column (1.0 X 30 cm) with 70% formic acid resulting in separation of eight major peptide peaks when monitored by absorbance at 280 nm. Fractions of each peak were pooled and freeze-dried. Each sample was dissolved in 0.1% trifluoroacetic acid and then subjected to HPLC on a µBondapack C₁₈ column (0.4 X 30 cm) with a linear gradient from 0 to 30% acetonitrile in 0.1% trifluoroacetic acid (20). Fractions of each major peptide peak were freeze-dried and used for chemical analysis.

Determination of Amino Acid Sequences—Purified samples of mDPP and sDPP (30 µg each) and CNBr cleavage peptides (about 5 µg each) were sequenced on an Applied Biosystems Inc. model 477A Gas-Phase Sequencer with an on-line model 120A phenylthiohydantoin derivative analyzer using the manufacturer's program (20, 21). Poly(A)* RNA Extraction and Fractionation—Total poly(A)* RNA was prepared from rat liver and fractionated by centrifugation on a sucrose density gradient (5-20%) as described previously (20). Identification of fractions containing the DPP mRNA was carried out by translation in vitro of the fractionated poly(A)* RNA in the reticulocyte-lysate system (22). Each fractionated RNA (2 µg/assay) was incubated in the reaction mixture at 25°C for 90 min with 50 µCi of [³⁵S]methionine, [³⁵S]-Labeled DPP was immunoprecipitated from the translated products analyzed by SDS-PAGE (9.0% gels) followed by fluorography as described (23).

cDNA Library Construction and Screening—The poly(A)* RNA fraction enriched with the DPP mRNA was used for construction of the following cDNA library. Double-stranded cDNA was prepared according to protocols described by Gubler and Hoffman (24) and methylated with EcoRI methylase. Hirt extracters were ligated to cDNA and digested with EcoRI. Linked cDNA was purified by chromato-
Fig. 1. Electrophoretic comparison of membrane and soluble forms of DPP. mDPP (lane 1) and sDPP (lane 2) were purified as described under “Experimental Procedures,” and the purified mDPP was further treated with papain (lane 3). Each sample was subjected to PAGE in the absence (A) or presence (B) of SDS. Gels in A were stained for enzyme activity using Gly-Pro-2-naphthylamine in combination with Fast Garnet GBC. An arrow indicates the top of the separating gels. Gels in B were stained for protein with Coomassie Brilliant Blue. Marker proteins (lane M) include myosin (M, 200,000), β-galactosidase (116,000), phosphorylase b (92,500), bovine serum albumin (66,000), and ovalbumin (45,000).

Fig. 2. Immunoprecipitation of DPP from translation products directed in vitro by rat liver poly(A)+ RNA. Poly(A)+ RNA was prepared from rat liver and translated in vitro in a reticulocyte lysate translation system with [35S]methionine in the absence (lane 1) or presence (lane 2) of dog pancreatic microsomes. [35S]-Labeled DPP was immunoprecipitated and subjected to SDS-PAGE (9.0% gels) followed by fluorography as described under “Experimental Procedures.” Molecular mass markers used (lane M) are the rat complement C3 α chain (115,000 Da), transferrin (78,000 Da), C3 β chain (66,000 Da), and α1-protease inhibitor (56,000 Da) which had been metabolically labeled with [35S]methionine (23).

stretch of hydrophobic amino acids from position 7, in contrast to that of sDPP. Taken together, these results suggest that mDPP as compared with sDPP has a hydrophobic NH2-terminal extension of about 4 kDa which, when removed by papain, results in the conversion of mDPP to sDPP.

In Vitro Translation and Fractionation of DPP mRNA—Poly(A)+ was prepared from rat liver and translated in vitro with [35S]methionine. A single component of 87 kDa (Fig. 2, lane 1) was obtained by immunoprecipitation with anti-DPP IgG from the products translated in the absence of dog pancreas microsomes, whereas a larger component of 103 kDa (lane 2) was obtained from the products translated in the presence of the microsomes. The latter form was converted to an 88-kDa form when treated with endo-β-N-acetylglucosaminidase H (data not shown). The results indicate that the 87-kDa precursor of DPP is glycosylated to the 103-kDa form in the presence of the microsomes. The difference in mass between the 103-kDa form and purified mDPP (109 kDa) may reflect further processing of its oligosaccharides from the high mannose to the complex type.

For enrichment of the DPP mRNA, the poly(A)+ RNA fraction from rat liver was subjected to centrifugation on a sucrose density gradient and fractionated (data not shown). Cell-free translation of each RNA fraction identified the location of the DPP mRNA, a fraction of which was used for construction of a cDNA library.

Isolation of Two Overlapping cDNA Clones for DPP—One portion of the λgt11 cDNA library (6 × 106 clones), without prior amplification, was screened with anti-DPP IgG. Of seven positive recombinant phages obtained, clone λCD5 had the longest cDNA insert (3.6 kb). This insert was found to contain an open reading frame coding for all six CNBr fragments of DPP but not for the NH2-terminal sequences of mDPP and sDPP (Figs. 3 and 4), indicating that clone λCD5 does not contain the total mRNA sequence. For further screening of the cDNA library, we prepared an EcoRI-BglII fragment of 891 base pairs of λCD5 as a probe. Screening of 8 × 106 clones with the new probe yielded 40 positive plaques, of which clone λCD37 had the longest insert, with 3.5 kb. The cDNA inserts of the two clones thus obtained were subcloned into the plasmid vector pUC118, and the relationship among these plasmid inserts was analyzed by restriction endonuclease mapping (Fig. 3). The clones λCD37 and λCD5 contained overlapping cDNA inserts which together spanned a stretch of 4.9 kb of DNA.

Nucleotide and Deduced Amino Acid Sequence of DPP—The two cDNA inserts were sequenced from multiple restriction sites and on both DNA strands according to the strategy outlined in Fig. 3. The combined nucleotide sequence includes the complete coding and 3'-noncoding region, as well as part of the 5'-noncoding region (Fig. 4). λCD37 comprises 3512 base pairs corresponding to nucleotides from -88 to 3424, in which an in-phase TGA stop codon is found 54 nucleotides upstream from the initiator ATG. λCD5 comprises 3576 base pairs corresponding to nucleotides from 1271 to the 3'-end at 4846, in which the polyadenylation signal AATAAA is found at two sites. The appearance of more than one polyadenylation signal may imply the occurrence of polymorphism in the 3'-noncoding region of mRNA.

Fig. 3. Restriction map and sequencing strategy for cDNA clones of DPP. The entire inserts of λCD37 and λCD5 and various fragments prepared by appropriate restriction enzyme digestions were subcloned into plasmid vectors pUC118 and pUC119 and sequenced as described under “Experimental Procedures.” Arrows indicate the direction and extent of each sequence determination. The protein coding region is indicated by a thick closed bar.
The open reading frame encodes a protein of 767 residues with a calculated mass of 88,107 Da (Fig. 4). The sequence starts with a putative signal peptide which has a hydrophobic core domain (indicated by a broken line in Fig. 4) preceded by the positively charged lysine. The predicted NH₂-terminal sequence, however, is found to be completely identical to that of mDPP determined by Edman degradation. In addition, the NH₂-terminal sequence for sDPP is identified in a sequence segment in any other portion is in agreement with the fact that papain treatment releases a completely soluble form of DPP from the membrane. Either or both of two relatively large hydrophobic domains (residues 394-411 and 636-657) may be involved in the process. Amino acid residues that are part of the consensus sequence Asn-X-Ser/Thr for N-glycosylation sites have been plotted with respect to positions in the amino acid sequence. The window used in the scanning was 11 amino acids.

DPP from the membrane. Either or both of two relatively hydrophobic domains (residues 394-411 and 636-657) may be related to formation of the DPP dimer for which no covalent linkage has been identified (2, 4).

The primary structure of DPP thus obtained also demonstrates that five of the eight potential N-glycosylation sites are characteristically observed in the middle portion of the sequence. Hydrophobicity values obtained according to Kyte and Doolittle (31) have been plotted with respect to positions in the amino acid sequence. The window used in the scanning was 11 amino acids. Regions of about 20 amino acids with a hydropathy average greater than 1.6 are likely to be associated with the lipid bilayer, spanning the membrane in a helical conformation (31, 36). The only potential hydrophobic anchor segment is the sequence between residues 7 and 28 at the NH₂-terminus (Figs. 4 and 5). The absence of a sufficiently hydrophobic segment in any other portion is in agreement with the fact that papain treatment releases a completely soluble form of DPP.

Fig. 5. Hydropathy plot of the rat liver DPP protein sequence. Hydrophobicity values obtained according to Kyte and Doolittle (31) have been plotted with respect to positions in the amino acid sequence. The window used in the scanning was 11 amino acids. Line segments above and below the horizontal axis indicate hydrophobic and hydrophilic portions, respectively. A long arrow indicates the papain cleavage site for release of sDPP. The membrane-spanning region (hatched box), potential N-linked glycosylation sites (filled "lollipops"), and positions of cysteine residues (small arrows) are also indicated on the stick diagram above the hydropathy plot. The only potential hydrophobic anchor segment is the sequence between residues 7 and 28 at the NH₂-terminus (Figs. 4 and 5). The absence of a sufficiently hydrophobic segment in any other portion is in agreement with the fact that papain treatment releases a completely soluble form of DPP.

Fig. 4. Nucleotide sequences of cloned cDNAs and deduced amino acid sequence of rat liver DPP. The deduced amino acid sequence is shown below the nucleotide sequence. Both nucleotides and predicted amino acids are numbered on the right. Amino acid sequences of DPP determined by Edman degradation are underlined. A broken underline indicates a stretch of hydrophobic amino acids, a possible transmembrane domain, in the NH₂-terminal region. An arrow indicates the papain cleavage site for release of sDPP. Boxed residues represent potential N-linked glycosylation sites. Glycosylation of some of these asparagine residues could account for the difference between the molecular mass calculated from the amino acid composition (88,107 Da) and that estimated by SDS-PAGE for the mature protein (109 kDa).
Homology to gp110—The predicted amino acid sequence of DPP was compared for homology with the known sequences of other membrane-bound enzymes and glycoproteins in rat liver. Striking homology was found between DPP and gp110, a bile canaliculus domain-specific membrane glycoprotein (37). As shown in Fig. 6, gp110 has the entire sequence of 792 residues in contrast to the 767 in DPP. However, only 5 amino acid residues are different from each other in the corresponding positions when compared up to position 767. The difference of 4 residues in positions 183, 394, 562, and 624 is caused by single nucleotide substitutions, and that in position 767 results from a single nucleotide insertion (indicated by an arrow in Fig. 6). The frame shift by this insertion also causes an extension of the open reading frame, resulting in the longer sequence in gp110.

DISCUSSION

Clones acDP37 and acD5 obtained in this study contain overlapping DNA inserts which together span a stretch of 4.9 kb of DNA. Complete sequence analysis of the clones demonstrates an open reading frame of 2301 nucleotides starting at the first ATG codon encountered from the 5′-end. We believe that this open reading frame encodes the total primary structure of DPP. This is based on the findings that the NH2-terminal sequence of the mDPP and several other sequences determined by Edman degradation are all identified in the protein sequence deduced from the cDNA and that an in-phase TGA stop codon is found 54 nucleotides upstream from the initiator ATG (Fig. 4).

An attempt to identify the membrane-anchoring domain of DPP was made previously by Macnair and Kenny (2). They purified the detergent and autolysis forms of DPP from pig kidney microvillar membrane and analyzed them for their NH2-terminal sequences. The sequences determined for the detergent (8 residues) and autolysis forms (16 residues) correspond to those starting at the 12th and 37th positions, respectively, from the NH2 terminus in the primary structure predicted here, although there are differences in some positions due to the species difference. All the data presented in this study support the conclusion that DPP is anchored in the membrane by a single hydrophobic segment located at the NH2 terminus: (i) the NH2-terminal sequence of the detergent form is completely identical to that of the predicted structure; (ii) the sequence between residues 7 and 28 at the NH2 terminus is the only domain that fulfills the criteria proposed by Kyte and Doolittle (31) for membrane-spanning domains.

Fig. 6. Comparison of the DPP sequence with that of gp110. The nucleotide and deduced amino acid sequence of rat liver DPP were compared with those of gp110, a bile canaliculus domain-specific glycoprotein of rat hepatocytes (37). Amino acid residues at positions 183, 394, 562, and 624 are different from each other, due to single nucleotide substitutions as indicated by vertical bars. In contrast to the 767-residue sequence in DPP, gp110 has a further extension to position 792, which is caused by a single nucleotide insertion (indicated by an arrow). The frame shift also results in an amino acid substitution at position 767.

In conclusion, the predicted amino acid sequence at every single nucleotide position of DPP indicates that it is a member of the signal peptidase IV family with three domains: a stalk between the predicted precursor and mature forms. It is, how-ever, unlikely that all of the eight sites are glycosylated since papain has an NH2-terminal sequence starting at position 25 in the predicted sequence; (iv) the difference in molecular mass between mDPP and sDPP (4 kDa) is in good agreement with the mass of the predicted fragment (7822 Da of residues 1–34) remaining in the membrane after papain cleavage.

The NH2-terminal segment of DPP is also the most probable candidate for a signal sequence to target the protein to the endoplasmic reticulum and initiate its translocation across the membrane. Thus this highly hydrophobic stretch is proposed to have dual roles as both a translocation signal and membrane anchor. An uncleaved signal sequence functioning as a membrane anchor at the NH2 terminus has been shown for influenza neuraminidase (38), γ-glutamyl transpeptidase (13, 14), sucrase-isomaltase (15), and neutral endopeptidase (EC 3.4.24.11) (16). There is no explanation available at present for why the NH2-terminal signal peptide of these membrane proteins is uncleaved, although mechanisms for protein insertion into membranes have been proposed by many investigators (for review see Refs. 39 and 40). A cleavable NH2-terminal signal sequence found on most secretory proteins and many transmembrane glycoproteins has three structurally and possibly functionally distinct regions: a basic NH2-terminal region, a hydrophobic core region, and a more polar COOH-terminal region (41–43). In the last region of the three, positions −3 and −1 relative to the cleavage site are considered to be the most important for recognition by signal peptidase (41, 42). Three regions (residues 1–6, 7–28, and 29–35) of the DPP sequence reasonably satisfy the criteria proposed for the three domains of the cleavable signal. Nevertheless, cleavage of the signal sequence does not occur in DPP, suggesting that there must be some difference between the cleavable and uncleavable signals. A careful comparison of the available sequences reveals that the hydrophobic core region of all the uncleavable signals consists of at least 22 amino acid residues (13–16, 38, and this study), significantly different from that of the cleavable ones with the average of 13 residues (variable from the shortest 7 to the longest 16) (41–43). Thus, it is likely that the length of the hydrophobic core region is an additional important factor for actual cleavage by signal peptidase.

The predicted DPP sequence has no cysteine residue in the NH2-terminal half of the molecule followed by a middle portion containing a relatively high content of cysteine residues (Figs. 4 and 5). This may be favorable for a "stalked" structure proposed for DPP (2, 3), the stalk of which is easily attacked by proteinases such as papain. Although a Ser/Thr-rich sequence, which could be potential sites for O-glycosylation, is characteristically observed in the stalked domain of sucrase-isomaltase (3, 15) and the low density lipoprotein receptor (44), the DPP structure contains no such sequence. In fact, no galactosamine was identified in purified DPP when analyzed for its chemical composition. The sequence, instead, contains eight potential N-glycosylation sites, glycans of which accounts for the difference in molecular mass between the predicted precursor and mature forms. It is, however, unlikely that all of the eight sites are glycosylated since detailed analysis of its carbohydrate moiety suggests the presence of at most six oligosaccharide chains/molecule (9).

Striking homology in the predicted sequences of DPP and gp110 (37) is of particular interest (Fig. 6). The gp110 glycoprotein was isolated from rat liver plasma membranes, the same source as our DPP preparation, and identified by an immunocytochemical technique to localize specifically in the bile canaliculus domain (45) as has been shown for DPP (5, 2). O. Ogata, T. Fujiwara, and Y. Ikehara, manuscript in preparation.
6). Our preparation of DPP was always monitored by the enzyme activity, and the polyclonal antibodies against the purified enzyme were found to be monospecific for it. In contrast, gp110 has no assigned enzymatic activity because it was purified as a denatured form from gels after SDS-PAGE (45). The anti-gp110 antibodies, however, were reported not to cross-react with DPP (45). It was speculated that a hydrophobic domain at the COOH terminus of gp110 (residues 624-648) is its transmembrane domain (37), in contrast to our proposal.

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REFERENCES
1. McDonald, J. K., and Schwabe, C. (1977) in Proteinases in Mammalian Cells and Tissues (Barrett, A. J., ed) pp. 371-376, North-Holland Publishing Co., Amsterdam
2. Macnair, R. D. C., and Kenny, A. J. (1979) Biochem. J. 179, 379-395
3. Semenza, G. (1986) Annu. Rev. Cell Biol. 2, 255-313
4. Elovson, J. (1980) J. Biol. Chem. 255, 5807-5815
5. Fukasawa, K. M., Fukasawa, K., Sahara, N., Harada, M., Kondo, Y., and Nagatsu, I. (1981) J. Histochem. Cytochem. 29, 337-343
6. Bartles, J. R., Braiterman, L. T., and Hubbard, A. L. (1985) J. Biol. Chem. 260, 12792-12802
7. Kenny, A. J., Booth, A. G., George, S. G., Ingram, J., Kershaw, J., Wood, E. J., and Young, A. R. (1976) Biochem. J. 157, 169-182
8. Fukasawa, K. M., Fukasawa, K., Hiraoka, B. Y., and Harada, M. (1981) Biochem. Biophys. Acta 657, 179-189
9. Yamashita, K., Tachibana, Y., Matsuda, Y., Katsumuma, N., Kochibe, N., and Kobata, A. (1988) Biochemistry 27, 5565-5573
10. Feracci, H., Maroux, S., Boncien, J., and Desnuelle, P. (1982) Biochim. Biophys. Acta 684, 133-136
11. Matsuda, Y., Tsuji, A., and Katsumuma, N. (1983) J. Biochem. (Tokyo) 93, 1427-1433
12. Fulcher, I. S., Pippin, D. J. C., and Kenny, A. J. (1986) Biochem. J. 240, 305-308
13. Laperche, Y., Bulte, F., Aissani, T., Chobert, M.-N., Aggerbeck, M., Hanoune, J., and Guellaen, G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 937-941
14. Coloma, J., and Pitot, H. C. (1986) Nucleic Acids Res. 14, 1393-1403
15. Hunziker, W., Spiess, M., Semenza, G., and Lodish, H. F. (1986) Cell 46, 227-234
16. Devault, A., Lazure, C., Nault, C., Le Moul, H., Seidah, N. G., Chretien, M., Kahn, P., Powell, J., Mallet, J., Beaumont, A., Roques, B. P., Crine, P., and Boileau, G. (1987) EMBO J. 6, 1317-1322
17. Ray, T. K. (1970) Biochim. Biophys. Acta 196, 1-9
18. Ikehara, Y., Takahashi, K., Mano, K., Eto, S., and Kato, K. (1977) Biochim. Biophys. Acta 470, 202-211
19. Yoshiimoto, T., and Walter, R. (1977) Biochim. Biophys. Acta 485, 391-401
20. Misumi, Y., Tashiro, K., Hattori, M., Sakaki, Y., and Ikehara, Y. (1988) Biochim. J. 249, 661-668
21. Ogata, S., Hayashi, Y., Takami, N., and Ikehara, Y. (1988) J. Biol. Chem. 263, 10493-10494
22. Pelham, R. B., and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247-256
23. Misumi, Y., Misumi, Y., Miki, K., Takatsuki, A., Tamura, G., and Ikehara, Y. (1986) J. Biol. Chem. 261, 11398-11403
24. Gubler, U., and Hoffman, B. J. (1983) Gene (Amst.) 25, 263-269
25. Young, R. A., and Davis, R. W. (1983) Science 222, 778-782
26. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, pp. 368-369, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Hayn, T. V., Young, R. A., and Davis, R. W. (1985) in DNA Cloning: A Practical Approach (Glover, D. M., ed) Vol. 1, pp. 49-78, IRL Press Ltd., Oxford
28. Vieira, J., and Messing, J. (1982) Gene (Amst.) 19, 259-268
29. Sanger, F., Nickleu, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5465-5467
30. Kuhara, S., Matsu, F., Futamura, S., Fujita, A., Shinohara, T., Takagi, T., and Sakaki, Y. (1984) Nucleic Acids Res. 12, 89-99
31. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105-132
32. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404-427
33. Laemmli, U. K. (1970) Nature 227, 680-685
34. Nagatsu, T., Hino, M., Fujimori, H., Haya-saka, T., Takagi, T., and Sakaki, Y. (1984) Nucleic Acids Res. 12, 89-99
35. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
36. Eisenberg, D. (1984) Annu. Rev. Biochem. 53, 595-623
37. Hong, W., and Doyle, D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7962-7966
38. Fields, S., Winter, G., and Brownlee, G. G. (1981) Nature 290, 213-217
39. Sabatini, D. D., Kreibich, G., Morimoto, T., and Adesnik, M. (1982) J. Cell Biol. 92, 1-22
40. Wickner, W. T., and Lodish, H. F. (1986) Science 230, 400-407
41. Perlman, D., and Halvorson, H. O. (1983) J. Mol. Biol. 167, 391-409
42. von Hejne, G. (1983) Eur. J. Biochem. 133, 17-21
43. von Hejne, G. (1985) J. Mol. Biol. 184, 99-105
44. Russell, D. W., Schneider, W. J., Yamamoto, T., Laskey, K. L., Brown, M. S., and Goldstein, J. L. (1984) Cell 37, 577-585
45. Petell, J. K., Diamond, M., Hong, W., Buja, Y., Amari, S., Pittschrieler, K., and Doyle, D. (1987) J. Biol. Chem. 262, 14753-14759