The Glycine Cleavage System

MOLECULAR CLONING OF THE CHICKEN AND HUMAN GLYCINE DECARBOXYLASE cDNAs AND SOME CHARACTERISTICS INVOLVED IN THE DEDUCED PROTEIN STRUCTURES*

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A cDNA encoding chicken glycine decarboxylase (pCP15b) was isolated using an antibody specific to this protein. Additional cDNAs were cloned with the aid of the genomic fragments obtained by using the pCP15b cDNA probe. No initiator methionine codon is found in the currently elucidated cDNA sequence, and an ATG codon in an exon is assigned to this role. The precursor glycine decarboxylase deduced from the 3514-base pair nucleotide sequence is comprised of 1,004 amino acids (Mₐ = 111,848). The 1,020 amino acid residues are encoded for the precursor form of human glycine decarboxylase (Mₐ = 112,869) in the 3,783-base long cDNA sequence of two 1.9-kilobase pair cDNAs with a pentanucleotide overlap.

The pyridoxal phosphate binding site lysine and a glycine-rich region, which is suggested to be responsible for the attachment of the phosphate moiety of pyridoxal phosphate, are found in close proximity in both the chicken and human enzymes. This region essential for the enzyme action is suggested to be embedded in a segment rich in β-turns and random coils and is surrounded by conserved and repetitive amino acid sequences. It is suggested that these structures are involved in the organization of the active site of glycine decarboxylase.

The glycine cleavage system (1) (glycine synthase (EC 2.1.2.10)) is a multienzyme system comprised of glycine decarboxylase (2) (tentatively known as P-protein due to the requirement for pyridoxal phosphate (1), or P₁ (3)), H-protein, T-protein, and lipoamide dehydrogenase and reversibly catalyzes the degradation of glycine in animal and plant mitochondria and in prokaryotes (1). Some interesting properties involved in the catalytic reaction have been well documented using both glycine decarboxylase and H-protein purified from chicken liver. Glycine decarboxylase is almost inactive by itself and forms an enzyme complex with H-protein, resulting in a spectral change of the prosthetic group, pyridoxal phosphate, and conversion to the active enzyme. It is physiologically significant that H-protein functions as a regulatory protein, in addition to transferring two electrons through the prosthetic lipoamide moiety in the reversible reaction (2, 4, 5).

Our long term goal is the elucidation of the mechanism for the synthesis of these two proteins and their mode of interaction. However, because neither the cloning of the glycine decarboxylase cDNA nor the structural analysis of this protein was available, these issues are still unclear. In the accompanying study (6), we cloned and characterized chicken H-protein cDNA. The reported primary structure of this H-protein confirms that previously determined by Fujiwara et al. (7) using the protein purified from chicken liver. We attempted to isolate the chicken glycine decarboxylase cDNA. If this attempt was successful, then the cDNA would serve as a start for realizing our goals listed above.

In vertebrates, the glycine cleavage activity exists mainly in the liver, kidney, and brain (8) and is the physiologically major pathway for the catabolic degradation of glycine (9). Impaired or defective breakdown of glycine causes nonketotic hyperglycinemia, an incurable disease in humans (10). Defective glycine decarboxylase is most frequently assigned as the primary cause of this disease (11). Knowledge of the human glycine decarboxylase cDNA would be indispensable to the further study of this disease. Taking advantage of the fact that the human enzyme is reactive to an antibody raised against the chicken enzyme (12), we tried to clone the cDNA encoding the human enzyme. In this paper, we report on the cloning of the chicken and human glycine decarboxylase cDNAs and some characteristics expected to be responsible for the action of these enzymes.

EXPERIMENTAL PROCEDURES

Materials—Livers from White Leghorn hens (about 10 months old) were used. Radioactive nucleotides were obtained from Du Pont-New England Nuclear, and Bluescript plasmid vector (Stratagene Cloning Systems) was used to subclone various DNA fragments. All other materials were commercially obtained.

Partial Primary Structures of Chicken Glycine Decarboxylase—Glycine decarboxylase purified from chicken liver as described previously (2) was carboxymethylated and digested with tosyl-L-phenylalanine chloromethyl ketone-treated trypsin (13). Nine peptides were randomly purified by reverse phase high performance liquid chromatography (14) and subjected to sequence analysis using an automated protein sequencer, model 470A with on-line facility for phenylthiohydantoin analysis, model 120A (Applied Biosystems Inc.). The purified chicken glycine decarboxylase was also subjected to amino-terminal sequence analysis by this method.

Although the recovery of phenylthiohydantoin amino acids was...
extremely low (5% relative to the amount of the subunit used (10 nmol)), the primary structure of a peptide consisting of 15 amino acids was determined. The summarized result is shown in Table I. The peptides designated as 6 and 7 were reverse-translated, in part, into synthetic oligodeoxynucleotides as the strand complementary to mRNA and with all the possible codon usages, using a DNA Synthesizer, model 381A (Applied Biosystems Inc.). They were designated as probes 6 and 7, and the nucleotide sequence for probe 6 is 5'-TTT(GA)AC(CI)CTGT(CI)(GGA)(CTCCAT-3' and for probe 7 is 5'-AC(GA)AA(CI)GG(CI)(GGA)(AA-3'. The inosine nucleotides, shown as I, replaced A, T, or G at the wobble sites as recommended by Ohtsuka et al. (15).

Selection of Chicken and Human Glycine Decarboxylase cDNAs and Their Genes—Several cDNA clones were selected from a chicken liver cDNA expression library (6) using an immunopurified anti-chicken glycine decarboxylase antibody (12), but only the 2-kb cDNA clone (pCP15b) hybridized to the end-labeled probes 6 and 7. None of the clones selected from this library with the pCP15b cDNA probe had cDNA longer than the pCP15b insert. In parallel, we cloned the chicken glycine decarboxylase gene from a chicken genomic library (6) using the pCP15b cDNA. The pCP101E4.5 insert, a subcloned EcoRI fragment of this gene, could hybridize to RNA with a size similar to that revealed by the nick-translated pCP15b insert. One of several immunoreactive cDNA clones from the primary selection hybridized to the nick-translated pCP101E4.5 insert and was subcloned (pCP23a). A commercial cDNA library (CLONTECH Laboratory Inc.) was used for screening with the pCP23a cDNA probe, and four additional cDNA clones were obtained and characterized (pCP110b, pCP112a, pCP104b, and pCP102a).

A human liver cDNA expression library (16) was screened with the same antibody used for the selection of the chicken cDNA, and human glycine decarboxylase cDNAs were isolated (λHGD34c and λHGD34d). The 5'-EcoRI site of λHGD34d cDNA had been altered, but the cDNA was subcloned with part of the vector DNA which includes a HindIII site. The pHGD34d cDNA was employed to clone λHGD62a from a λgt10 human liver cDNA library. The pHGD34d and pHGD52a cDNAs hybridized to the pCP15b insert and the pHGD34c cDNA to the pCP23a insert.

Human high molecular weight DNA was prepared from the nucleated blood cells of a normal male by the method of DiLeIla and Woo (17). A partial digest of genomic DNA with Sau3AI was subjected to construction of a human genomic library with λX-DASH DNA (Stratagene Cloning System) according to the method recommended (18). A genomic clone (λHGDG27) was obtained using the nick-translation pHGD34c and pHGD34d cDNAs and partially characterized. Throughout the screening, the probing was conducted as described in the preceding paper (16). Restriction maps and relative locations of the chicken and human cDNAs are presented in Figs. 1 and 2.

DNA Sequencing—The pCP121a0.5 insert, a 0.5-kb stretch between the 5'-end EcoRI and EcoRV sites of pCP112a, was treated with PvuII and AcII, and the resultant fragments (120–270 bp) were subcloned. The 2.0- and 0.9-kb fragments formed from pCP110b by EcoRI were subcloned (pCP110b.2 and pCP110b.9). The pCP110b.2 insert was subjected to isolation of serial deletion mutants for both strands as described in the preceding paper (19). The Smal, Xhol, and PstI fragments of the pCP110b.9 insert was subcloned.

For the human glycine decarboxylase cDNA, a similar sequencing strategy was undertaken. Serial deletion mutants of both strands were prepared from the pHGD34c cDNA. Several fragments of about 200–280 bp were formed from the pHGD34d, pHGD15a, and pHGD52a inserts by treating them with EcoRV, Apal, EcoRI, Smal, Sau3AI, and Alul, and then subcloned.

Nucleotide sequencing (20) was performed using the promoter sequences for T3 and T7 RNA polymerases on the plasmids listed above as priming sites, [α-32P]dATP and 7-deaza-dGTP (21). Both the nucleotide sequences and the deduced primary structures were analyzed using a computer program, MicroGenie, which was developed by C. Queen and L. Korn (Beckman Instruments, Inc.). Chicken liver poly(A)+ RNA (6) and human liver total RNA (16) were used for Northern analysis (22).

RESULTS

Characterization of Chicken Glycine Decarboxylase cDNA—The chicken glycine decarboxylase subunit exhibits a molecular mass of about 100 kDa (905 amino acids (2)), and the expected size of mRNA encoding this protein is at least 2.7 kb. The pCP15b cDNA, only the clone identified with the specific antibody and synthetic oligonucleotide probes, was

![Image](https://example.com/image1.png)

**Fig. 1** Restriction map and strategy employed to sequence chicken glycine decarboxylase cDNA. A, the restriction sites determined by the digestion with restriction endonucleases and confirmed in the nucleotide sequence are indicated along the protein coding (open bar) and untranslated (closed bar) regions. B, the relative locations in the cDNA sequence of the cDNA clones are shown. C, each arrow indicates the length and direction of the sequence determined.

**Table I**

| Amino acid sequence | Position in the deduced structure |
|---------------------|----------------------------------|
| A                   |                                  |
| H-D-F-C-R           | 50-55                            |
| N-L-E-N-A-G-W-V-T   | 140-149                          |
| D-I-S-L-V-H-S-M-I-P-L-G | 529-540                        |
| A-Y-L-N-A-K         | 615-620                          |
| D-A-C-P-L-G         | 777-782                          |
| M-D-P-Q-V-N-P-L-K   | 922-930                          |
| E-V-A-A-K-F-L-P-V-K | 951-961                          |
| F-W-P-T-P-L-R       | 966-972                          |
| I-D-I-Y-G-D-Q-H     | 973-981                          |
| B                   |                                  |
| G-G-E-A-A-R-C-I-E-Q-L-L-P-R | 35-49                          |
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**Fig. 2.** Restriction map and strategy employed to sequence human glycine decarboxylase cDNA. A, the restriction sites determined and confirmed by the same way as described for Fig. 1 are shown along the protein coding (open box) and untranslated (closed box) regions. B, the relative locations of the representative clones are shown. The junction of the pHGD34c and pHGD34d cDNAs is located at nucleotides 1,873-1,877 (see Fig. 4C). Each arrow indicates the length and direction of the sequence determined.

**Fig. 3.** The sizes of the chicken and human glycine decarboxylase mRNAs. Chicken liver poly(A)+ RNA (5 µg, for lane 1) and human liver total RNA (15 µg, for lane 2) were denatured and subjected to Northern analysis. The denatured λcl87Sam7 DNA (StyI digest) and E. coli ribosomal RNA were used as size markers.

less than 2 kb in size, although this cDNA probe reveals a 4-kb mRNA (Fig. 3). Additional clones, pCP110b and pCP112a, were isolated by using the pCP23a cDNA which was obtained with the aid of a genomic fragment cloned with the pCP15b cDNA. The pCP110b cDNA had a 15-bp poly(A) region and hybridized to the pCP15b at the 3′ region. The other side of this cDNA hybridized to the pCP23a cDNA, and the pCP112a cDNA encodes an approximately 100-bp long sequence upstream of the pCP23a cDNA (cf. Fig. 1). Nucleotide sequences, determined by using these inserts and primary structure deduced for chicken glycine decarboxylase are shown in Fig. 4, A and B. This 3,490-bp long cDNA contains the sequence for the amino-terminal primary structure shown in Table I, B (nucleotides 79-123), but no initiator methionine codon in the upstream reading frame. All of the nine tryptic peptides can be located in the primary structure deduced from an identical reading frame (underlined in Fig. 4B), indicating that the selected cDNAs are those for chicken glycine decarboxylase.

An exon in a genomic subclone, pCPG301EE2.9, includes nucleotides 1-201 of the cDNA. An ATG codon was found 24 bp upstream on an identical genomic stretch. This ATG codon is included in the reading frame encoding a peptide of 34 amino acids followed by the amino-terminal peptide of the purified protein (Fig. 4A) and assigned to the role of the initiator methionine codon. Two additional ATG codons (275 and 298 bp upstream) were out of this frame. The precursor glycine decarboxylase comprised of 1,004 amino acids ($M_r = 111,848$) ends with a translation termination codon (nucleotides 3,013-3,015), and two AATAAA motifs are at nucleotides 3,338-3,343 and 3,481-3,486.

**Characterization of Human Glycine Decarboxylase cDNA—** Two 1.9-kb cDNAs were cloned and distinguished by the restriction mapping (pHG34c and pHGD52a in Fig. 2). The pHGD34c cDNA is 1,873 bp in size (see Fig. 4C) and shows no significant hybridization with both the pHGD52a and pHGD34d cDNAs, although these cDNAs seem to hybridize to a single RNA (Fig. 3). The first ATG codon begins at nucleotide 151 and precedes an open reading frame encoding 574 amino acids. However, the sequences at the 3′-end of the pHGD34c cDNA, AAAGAATT (1,870-1,877 in Fig. 4C) and 5′-end of the pHGD34d cDNA (GAATTTGC, nucleotides 1,672-1,682 in Fig. 4B), and the two individuals reading frames of the immunoreactive pHGD34c cDNA, pHGD34d cDNAs are not changed.

Analysis of the genomic structure confirmed that this junction sequence is actually coded in an exon in a genomic clone, λHGDG27. A XbaI fragment from this clone was selected by hybridization with both a 3′ region of the pHGD34c cDNA and a 5′ region of pHGD34d cDNA. An exon near the 3′-end of this XbaI fragment is comprised of a 143-bp sequence, and, thereby, the 5′-6-bp long sequence from the 3′-end of the pHGD34d cDNA links consecutively to the remnant part corresponding to the 5′-end of the pHGD34d cDNA (Fig. 5). This result indicates that there are probably no sequences inserted between the two sequences determined from the pHGD34c and pHGD34d or pHGD52a cDNAs. The precursor form of human glycine decarboxylase deduced from the 3783-bp human glycine decarboxylase cDNA sequence is composed of 1,020 amino acids ($M_r = 112,869$), and the first translation termination codon and a consensus for poly(A) site are located at nucleotides 3,211-3,213 and 3,743-3,748, respectively.

Some Characteristics of the Structure of Glycine Decarboxylase—The primary structure of the human enzyme was aligned over that of the chicken enzyme at the peptides
Fig. 4. The nucleotide sequences of the cDNAs encoding the chicken and human glycine decarboxylases and their deduced primary structures. A, the partial nucleotide sequence of an exon for the amino-terminal region of the chicken precursor glycine decarboxylase is determined in the pCPG301ERE2.9 insert and shown. B, the nucleotide sequence of the chicken glycine decarboxylase cDNA and the deduced primary structure are shown. Numbering for the nucleotides and amino acids begins at A of the putative initiator methionine codon in the genomic sequence shown in A and, therefore, at this methionine. The primary structures of the peptides shown in Table I are underlined. The positions of the synthetic probes 6 and 7 are indicated with the double underlines. Asterisks show the translation termination codon, and the two poly(A) signals are indicated with a line over the sequence. C, a nucleotide sequence of the human glycine decarboxylase cDNA and the deduced primary structure are shown. The translation termination codon and a consensus for poly(A) site are indicated with the asterisk and double underlines, respectively. The extended and conserved Ser-rich sequences are boxed, and the Gly-rich region is underlined (cf. text).
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FIG. 4—continued

beginning at Met\(^1\), because the amino-terminal regions of both enzymes (Met\(^1\) to Gly\(^9\)) are very similar (Fig. 4, B and C). The major difference is observed in the sequences from Leu\(^{10}\) to Leu\(^{12}\) of the human enzyme and from Arg\(^{11}\) to Leu\(^{12}\) of the chicken enzyme. The human enzyme, however, contains a glycine- and alanine-rich segment similar to the amino terminal region of the purified chicken enzyme, suggesting that the amino terminus of the human enzyme is probably Gly\(^{40}\), and that the majority of the difference involved is in their mitochondrial presequences. The distinct insertion of a heptapeptide, V-V-Q-T-R-A-K\(^-\) (Val\(^{227}\)-Lys\(^{238}\)), is found in the human enzyme, instead of Asn\(^{227}\) of the chicken enzyme. The carboxyl-terminal side from this position on (about 800 residues) contains no gap structure.

Overall, the primary structures of these two enzymes appear to be highly conserved. Structural homology was estimated to be 83.8% in putative forms of the mature enzymes. By taking into account the fact that these enzymes contain many amino acid substitutions between Asp and Glu, Arg and Lys, and Ser and Thr, and within branched-chain amino acids, the structural similarity is estimated to be near 93%. The carboxyl-terminal side is closely conserved. A dot matrix comparison of these two cDNA sequences, in which comparison of segments consisting of 8 consecutive nucleotides that contain 6 identical nucleotides gave a dot, also showed a line in the coding regions for the mature protein (data not shown). This result is confirmatory of the finding that the human cDNAs were identified by hybridization to the chicken cDNAs under highly stringent conditions.

Repetitive amino acid sequences are commonly found in both the chicken and the human enzymes. A nonapeptide, ILSTPFKRT, is found in the chicken enzyme (amino acids 492-500), and its counterpart appears to be repeated as ILTDTRPFKKT (amino acids 848-857). The additional repetitions, SSAELAPISW and SSAILPISW (amino acids 548-557 and 791-799), are surrounded by the first nonapeptide repetitions. The human enzyme also contains SSSELAPITW.

Fig. 5. The genomic sequence of the exon coding for the junction of the pHGD34c and pHGD34d cDNAs. The 1.5- and 1.7-kb XbaI fragments of the XHGDG27 insert were selected by hybridization with the 3'-end BamHI/EcoRI fragment of the pHGD34c cDNA, and with the 5'-end HincIIIEcoRV fragment of the pHGD34d cDNA, and truncated with BamHI and EcoRV. The result obtained is shown with the short overlap between the pHGD34c and pHGD34d cDNAs (asterisks over the sequence). The 5' and 3' intron sequences (lower case letters) and the internal EcoRV site are also shown. Numerals indicate the positions of nucleotides including the intron sequences shown.

A.

![A. Diagram](image)

B.

![B. Diagram](image)

Fig. 6. Secondary structure predicted for the chicken and human glycine decarboxylases. A, positions of \(\alpha\)-helices in the deduced primary structures are indicated with closed boxes. Capital K over the bar expresses the active site lysine, and the glycine-rich region is shown by the hatched box. Arrows and numerals indicate the positions of the repetitive sequences and amino acids, respectively. B, the primary structure between Lys\(^{11}\)-Thr\(^{12}\) of the chicken enzyme (upper case letters) is shown with the predicted secondary structure (lower case letters; \(\alpha\), \(\alpha\)-helix; \(b\), \(b\)-structure; c, random coil; and \(\beta\), \(\beta\)-turn). Asterisks indicate the Lys\(^{11}\) and glycine-rich region.
The overall homology of the putative mature enzymes is estimated to be about 84%. The arrangement of α-helix supports the active site lysine and the glycine-rich region are embedded in a peptide rich in β-turns and random coils. These flexible structures might be responsible for the spectral change resulting from the interaction of glycine decarboxylase and H-protein (4). Neither rabbit serine hydroxymethyltransferase (which also binds glycine) nor other amino acid decarboxylases contain a similar glycine-rich region at positions adjacent to their active site lysine (32, 33). It is conceivable that the conserved repetitive sequences might have a specific function necessary for the enzyme activity, because their unique structures are present in the enzymes from the different organisms. This prediction remains to be examined.

Acknowledgments—We thank Prof. Keiya Tada, the Department of Pediatrics, Tohoku University School of Medicine, and Dr. Eiji Tsukamoto, Tsukamoto Hospital, for their encouragement.

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