Cloning and Nucleotide Sequence of Wild Type and a Mutant Histidine Decarboxylase from *Lactobacillus* 30a*

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Peter Vanderslice, William C. Copeland, and Jon D. Robertus

From the Clayton Foundation Biochemical Institute, Department of Chemistry, University of Texas at Austin, Austin, Texas 78712

Prohistidine decarboxylase from *Lactobacillus* 30a is a protein that autoactivates to histidine decarboxylase by cleaving its peptide chain between serines 81 and 82 and converting Ser-82 to a pyruvoyl moiety. The pyruvoyl group serves as the prosthetic group for the decarboxylation reaction. We have cloned and determined the nucleotide sequence of the gene for this enzyme from a wild type strain and from a mutant with altered autoactivation properties. The nucleotide sequence modifies the previously determined amino acid sequence of the protein. A tripeptide missed in the chemical sequence is inserted, and three other amino acids show conservative changes. The activation mutant shows a single change of Gly-58 to an Asp. Sequence analysis up- and downstream from the gene suggests that histidine decarboxylase is part of a polycistronic message, and that the transcriptional promoter region is strongly homologous to those of other Gram-positive organisms.

The function of histidine decarboxylase is to carry out the reaction histidine → histamine + CO₂. This activity is widespread in nature, and the histamine product often has powerful physiological effects on higher organisms. Many histidine decarboxylases, and indeed other amino acid decarboxylases as well, require pyridoxal phosphate as a cofactor (1). There exists a large subclass of enzymes, however, that use a group symmetry. That is, three chains form a trimer with a deep central cavity containing three active sites, and two of these trimers are then arranged tail to tail to form the hexamer (4). The μ chains are inactive but are capable of activating themselves by carrying out an autocatalytic chain cleavage between Ser-81 and Ser-82 (6). The amino-terminal 81 residues of the activated protein are referred to as the β chain and the larger fragment is the α chain. In the active protein, the amino-terminal serine of the α chain, Ser-82, has been converted to a pyruvoyl moiety, which then serves as the prosthetic group for the decarboxylation reaction. Snell and co-workers (7) have proposed several possible mechanisms for the chain cleavage and conversion of Ser-82 to a pyruvoyl moiety. They have shown that mechanism involves direct or indirect attack of the Ser-82 hydroxyl on the 81–82-peptide bond; Ser-82 probably undergoes an α,β elimination reaction followed by imine formation, hydration, and deamination to yield the pyruvoyl moiety. Several functional groups on the protein must participate in this autoactivation, but none has yet been positively identified. Once activated, histidine decarboxylase has a pH optimum of ~4.8 with a *Kₚ₅* for histidine of 0.4 mM and a turnover number of 3000 min⁻¹ (8).

In addition to the wild type histidine decarboxylase, Reesel and Snell (9) have isolated a number of mutants that produce partially active or inactive enzymes. The best studied of these is mutant 3, which produces a full-length protein that, unlike wild type, is inactive when isolated, and must be incubated at pH 7 to activate. The resulting protein, although exhibiting chain cleavage and pyruvoyl formation, is catalytically inactive unless the pH is lowered toward the optimum value of 4.8 (10).

Snell and co-workers have carried out the chemical sequence analysis of wild type and part of the mutant protein. The β chain has been sequenced for both proteins (11), and the amino acid sequence of the α chain for wild type has also been completely determined (12). The α chain of the mutant 3 protein has been inferred from preliminary sequence analysis to be identical with that of the wild type (12). The only differences seen between the proteins were in the β chain, where Ser-51 in the wild type was replaced by an Ala, and Gly-58 by an Asp (11).

In this paper we report the isolation of the genes for both proteins, their cloning, complete sequence analysis, and comparison with the amino acid sequence. Our efforts are aimed at expressing these proteins in *Escherichia coli* and carrying out site-directed mutagenesis of the protein to analyze further the autoactivation process and mechanism of catalysis.

**EXPERIMENTAL PROCEDURES**

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† To whom correspondence should be addressed.

| The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J02613.

‡ To whom correspondence should be addressed.
RESULTS AND DISCUSSION

Two mixed oligonucleotide probes based on the amino acid sequence of histidine decarboxylase (11, 12) were used to help in restriction mapping of the DNA around the histidine decarboxylase gene. A 20-base probe bound to regions of the gene coding for residues 27–33 near the amino terminus, whereas a 17-base probe bound the carboxyl-terminal region residues 245–250. A schematic of key restriction sites is shown in Fig. 1 (Appendix).

Initially we attempted to clone both the 6.7-kilobase pair EcoRI fragment and the 3.6-kilobase pair PstI-EcoRI fragment, which were known to contain the entire histidine decarboxylase gene and its upstream control elements. This effort proved futile, even when the appropriate DNA fractions were isolated from agarose gels before recombination in plasmids. A screening of over 4000 such clones by mixed probe hybridization revealed that none contained the histidine decarboxylase gene. We were forced to conclude that the gene, under control of its own promoter and in a high copy number plasmid, was lethal to the E. coli host cells. As a result, we began to clone a series of restriction fragments designed to allow the piecewise sequence analysis of the histidine decarboxylase gene.

The first fragment of the gene to be cloned and subjected to sequence analysis was the 650-base BamHI-EcoRI fragment, which specifies the carboxyl-terminal portion of the protein, and which was readily selected with the 17-base mixed oligonucleotide probe. The fragment was initially placed in a pUC8 plasmid for screening and then moved to M13 phage, strains mp18 and mp19, for sequence determination. The sequence of the template strand was determined in mp18 using the universal primer to read roughly 350 bases of sequence. This sequence overlapped the region binding the 17-base mixed probe. The mixed probe was then used as a second primer. It was possible to confirm roughly 70 bases previously determined and to continue 300 bases downstream to within 60 bases of the end of the fragment. The sequence revealed that the BamHI-EcoRI fragment specified the carboxyl-terminal 124 residues of the enzyme, as well as ~300 downstream bases. The sequence of the information strand was then determined in mp19, again by use of two primers. The downstream region was read from the universal primer, and the remainder read from a unique 19-base primer designed from the previously determined template strand. In this way, the nucleotide sequences of both DNA strands of the fragment were determined over the entire length corresponding to the histidine decarboxylase gene, and all of the downstream bases were read at least once.

The 2.8-kilobase pair PstI-BamHI fragment was cloned using pUC9 as the vector. Attempts to move this PstI-BamHI fragment containing the histidine decarboxylase promoter into M13 for sequence determination were not successful. This clone was confirmed to contain the histidine decarboxylase gene by double-stranded sequence analysis on the plasmid as described under "Experimental Procedures." Using our histidine decarboxylase mixed 20-mer as a primer for double-stranded sequence analysis, we were able to find key restriction sites allowing the gene fragment to be subcloned into M13.

Additional restriction fragments were subsequently cloned, and the sequence was determined in such a fashion as to cover both strands of the entire gene. The overall strategy is shown schematically in Fig. 2 (Appendix), in which arrows indicate the regions and direction of sequence analysis. The nucleotide sequences of the wild type and mutant 3 genes were independently determined by essentially identical strategies.

The Gene Sequence for Histidine Decarboxylase—Fig. 3 (Appendix) shows the base sequence of the information strand of DNA around the histidine decarboxylase gene. The sequence data begin in the control region upstream of histidine decarboxylase, proceed through the gene, continue on into a spacer region, and pick up a second open reading frame. The amino acid sequences corresponding to histidine decarboxylase and this second presumed protein are written above the gene sequence.

It is clear that histidine decarboxylase begins with a single methionine residue which is processed away, leaving a serine as the amino-terminal residue. The processed protein is 310 amino acids long, in contrast to the 307 residues reported from the chemical sequence analysis. The reason for this discrepancy is that a tripeptide cyanoethyl bromide fragment was evidently missed in the chemical sequence. This tripeptide corresponds to the Glu283–Met285 of our sequence.

In addition to the tripeptide insertion, we find only three minor discrepancies between our sequence and the chemical sequence. These are at position 206 where our Asn was reported as Asp, at 265 where Leu was reported as Ile, and at 270 where Ile was reported as Leu.

We have examined the codon usage frequency readings for histidine decarboxylase, and the result is shown in Table I. Like other organisms, Lactobacillus 30a appears to use a subset of roughly 25 of the 61 possible codons. The strong use of the initiation codon is AGGAGG. This is the consensus for the Shine-Dalgarno sequence of Gram-positive cells (27), as well as for E. coli, and presumably plays the same role in Lactobacillus. This sequence is enclosed by a box in Fig. 3.

Lactobacillus is a Gram-positive bacterium, and Graves and Rabinowitz (28) have recently completed a survey of the nucleotide sequence of the wild type histidine decarboxylase gene.

The initiation codon is included.

| Codon usage in Lactobacillus 30a wild type histidine decarboxylase gene |
|---------------------------|-----------------|-----------------|-----------------|
| Codon         | Frequency %    | Codon         | Frequency %    | Codon         | Frequency %    |
| UUU           | 7              | UCU            | 5              | ACC            | 6              |
| UUC           | 1              | UCG            | 1              | AAA            | 3              |
| UUG           | 1              | UCA            | 1              | GAC            | 2              |
| UAU           | 8              | CCA            | 4              | CAC            | 3              |
| UAG           | 5              | CCA            | 1              | CAG            | 3              |
| UGA           | 1              | CCA            | 0              | CAA            | 2              |
| UGA           | 0              | CCA            | 0              | CGA            | 1              |
| UAG           | 1              | CCA            | 0              | CCA            | 1              |
| UUG           | 1              | CCA            | 0              | CCA            | 0              |
| UAU           | 2              | CCA            | 0              | CCA            | 0              |
| UAC           | 0              | CCA            | 0              | CCA            | 0              |
| UAG           | 0              | CCA            | 0              | CCA            | 0              |
| UUG           | 0              | CCA            | 0              | CCA            | 0              |
| UAU           | 0              | CCA            | 0              | CCA            | 0              |
| UAC           | 0              | CCA            | 0              | CCA            | 0              |
| UAG           | 0              | CCA            | 0              | CCA            | 0              |
| UUG           | 0              | CCA            | 0              | CCA            | 0              |
| UAU           | 0              | CCA            | 0              | CCA            | 0              |
| UAC           | 0              | CCA            | 0              | CCA            | 0              |
| UAG           | 0              | CCA            | 0              | CCA            | 0              |
| UUG           | 0              | CCA            | 0              | CCA            | 0              |
| UAU           | 0              | CCA            | 0              | CCA            | 0              |
| UAC           | 0              | CCA            | 0              | CCA            | 0              |
| UAG           | 0              | CCA            | 0              | CCA            | 0              |
| UUG           | 0              | CCA            | 0              | CCA            | 0              |
| UAU           | 0              | CCA            | 0              | CCA            | 0              |
| UAC           | 0              | CCA            | 0              | CCA            | 0              |
| UAG           | 0              | CCA            | 0              | CCA            | 0              |

The initiation codon is included.
FIG. 4. Autoactivation of wild type and mutant histidine decarboxylase. The autoactivation of the enzyme was monitored as the presence of decarboxylase activity, represented here as the release of $^{13}CO_2$. A dose response is shown to increasing amounts of crude lysate from wild type and mutant Lactobacillus 30a. Both strains were incubated at 37°C for 24 h at pH 4.8 and 7.6. The lysates were brought to pH 5.2 for assaying. Wild type enzyme is represented as circles; the mutant is represented as triangles. Closed figures were preincubated at pH 4.8 and open figures at 7.6. The dose-response units are based on total lysate protein. One mutant unit corresponds to 0.5 µg of total protein, whereas the wild type was 2.5 µg.

Promotor sites of Gram-positive cells. Their tabulations are based only on those systems for which the control regions have actually been identified by chemical means. They report a consensus Pribnow box sequence, TATAAT, running 8-13 bases before the first transcribed base. We find the identical sequence lying 89-94 bases upstream of the initiator codon. For example, we see an A at position -24 which is conserved in more than 50% of Gram-positive control regions. We also see a pair of Ts at the -34 and -35 region, conserved in more than 75% of Gram-positive sequences and also seen in E. coli sequences. Finally, we see an A-rich region running from -45 to -41 conserved in more than half of Gram-positive organisms.

Downstream Sequence—The histidine decarboxylase gene terminates with the codon TAA. In addition, there are stop codons in both alternative reading frames just beyond the end of the gene. A TAG codon begins 12 bases beyond the enzyme amber codon and a TGA codon begins 17 bases beyond the gene. This arrangement prevents read-through to downstream genes, should there be out of frame expression in the histidine decarboxylase gene region, and is characteristic of polycistronic messages.

We have no chemical information linking the histidine decarboxylase expression to any other proteins. However, we do observe that beginning 80 bases downstream from the enzyme terminator is an open reading frame corresponding to 67 amino acids beginning with a Met. This sequence reaches to the end of the DNA fragments we have analyzed and may be part of a complete protein. Situated 13-7 bases upstream of the putative second initiator is the sequence AGGATT, which is a reasonable Shine-Dalgarno sequence. We see no sequence between the second open reading frame and the histidine decarboxylase gene that appears to be an acceptable promotor site. It is possible, therefore, that this second protein is a part of a histidine decarboxylase operon, although transcription and expression levels of the second protein may be regulated by other factors. For example, we observe that the space between the histidine decarboxylase gene and the second gene contains a palindromic sequence corresponding to an 8-base pair stem and 14-base loop structure. This structure may serve, for example, as a pause site for RNA polymerase, and, together with termination factors, may allow only a certain fraction of mRNA to be extended to the second protein, although we have no evidence to support such a contention. The stem region is underlined by arrows in Fig. 3.

The Mutant Histidine Decarboxylase Sequence—The base sequence of the mutant histidine decarboxylase gene, as well as up- and downstream regions, is identical to that of wild type with one exception. The wild type codon for Gly-58 is changed from GGT to Asp, GAT. This result is somewhat surprising since the chemical sequence of mutant 3 histidine decarboxylase was clearly determined to contain two mutations—the Gly to Asp at position 58, but also a Ser to Ala at position 51.

We isolated three other independent mutant clones and examined the sequence in this region, and they, too, showed only the position 58 change. Since our strain appeared to differ from the strain originally characterized by Snell and co-workers, a question arose as to the phenotype of our mutant. The Lactobacillus 30a strain we used was grown up the histidine decarboxylase fraction isolated as described under "Experimental Procedures." The sample was split into two, and one half was kept at pH 4.8 and the second brought to pH 7.6. Both samples were then incubated at 37°C for 24 h, and the pH 7.6 sample was brought back to pH 4.8 for the enzymatic assay. A mutant phenotype should not activate unless the pH is raised. Fig. 4 shows a dose-response curve for the two protein samples, as well as for a wild type control. The mutant histidine decarboxylase kept continuously at pH 4.8 has only background levels of histidine decarboxylase activity even at very high protein levels, whereas the higher pH half shows very strong activity and must have autoactivated. Wild type enzyme is active regardless of incubation history. Although we have not yet quantified our protein levels to make direct comparisons with the characterization of mutant 3 histidine decarboxylase, it is quite clear that this Lactobacillus 30a strain, the Asp-58 mutant, behaves as an autoactivation mutant. Since this strain may differ slightly from Snell's original mutant 3, we will refer to the enzyme derived from it as histidine decarboxylase mutant D58. The x-ray structure of histidine decarboxylase (4) shows that position 58 is on the outside of an α helix removed from the Ser1-Ser2 site by more than 3 helical turns in space, and makes no direct contact with it. As a result, if the mutational activation phenotype is due to this mutation in the protein,
the effect must be a subtle one, acting through forces applied to the protein chain (30). On the other hand, it may be that the mutant strain contains other cellular changes, not within the enzyme gene, that alter the activation rate, perhaps by affecting intracellular pH or ion levels.

**FUTURE PLANS**

We have cloned fragments and determined the complete sequence of the gene and control regions for wild type histidine decarboxylase and an autoactivation mutant. Our goal is to express the proteins in E. coli, which has no histidine decarboxylase activity of its own. Thereafter we plan to make the sequence of the gene and control regions for wild type histidine decarboxylase and an autoactivation mutant. Our goal is to affect intracellular activation scheme. It appears that the histidine decarboxylase promotor is too strong to allow viable clones to be made.

We have cloned fragments and determined the complete sequence determined from unique primers based on previously obtained physiological and control mutant. Our goal is to express the proteins in E. coli, which has no histidine decarboxylase and an autoactivation mutant. Our goal is to affect intracellular activation scheme. It appears that the histidine decarboxylase promotor is too strong to allow viable clones to be made. Graves and Rabinowitz (28) have noted that Gram-positive organisms tend to have strong promoters relative to those in E. coli, and that this problem of lethality is not infrequent. We plan to reassemble the gene under a more temperate, inducible promotor, and this procedure should allow expression of the enzyme.

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**APPENDIX**

**Fig. 1.** The restriction map around the histidine decarboxylase gene. The base line represents Lactobacillus DNA. The hatched box represents the coding region for the enzyme, with the amino-terminal coding regions on the left. The 3.6-kilobase pair EcoRI-PstI fragment on the extreme left is not to scale. Restriction endonuclease sites are indicated above the DNA. Kb, kilobase.

**Fig. 2.** The gene sequencing strategy for histidine decarboxylase. The line represents DNA near the gene. Restriction sites are H = Hind III, D = DraI, Hc = Hinc II, B = BamHI, and E = EcoRI. The hatched box represents the coding region for the enzyme, with the amino-terminal terminals to the left. The arrows show the direction and extent of gene sequencing from a variety of primers. Sequences determined from the universal primer begin at restriction sites and are single-headed arrows. Double-headed arrows show sequence determined from unique primers based on previously obtained sequence. The saw-toothed arrows show regions where sequence was determined from double-stranded pUC plasmids.
FIG. 3. The gene and amino acid sequence of histidine decarboxylase. The base sequence of the message-like information strand around histidine decarboxylase is shown as lines of capital letters, running 5' toward 3'. Within protein-coding regions, the bases are clustered as codons, and the amino acids written above in bold type. AMB marks the amber stop codon. The amino acids are labeled at 20-residue intervals. Beginning 80 bases downstream of the histidine decarboxylase gene is a second open reading frame. Seven bases upstream of each coding region is a presumed Shine-Dalgarno sequence enclosed in a box. The underlined box further upstream of the histidine decarboxylase gene is the presumed Pribnow box, whereas the residue with the asterisk is the presumed origin of transcription. Palindromic base sequence capable of producing stem and loop structures are indicated by mirrored arrows beneath the base sequence.
Nucleotide Sequence of Histidine Decarboxylase

Construction of Partial Segments - L. 1. The wild type and mutant 3 DNA were set with the modified restriction enzymes. Appropriate-sized fragments were easily from 3.88 kbp using each amplis after electrophoresis. The gel fracrions containing the gene fragments were usually verified by Southern blot analysis.

The fragments were isolated into appropriate restriction DNA plasmid DNA vector. Insert molecular analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. DNA nucleotide analysis was carried into a mucosal gel, were isolated, from nucleotides in a mucosal gel. 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