Histidine decarboxylase (HisDCase) from Lactobacillus buchneri was purified to homogeneity. Its subunit structure, \((\alpha\beta)_6\), and enzymatic properties resemble closely those of the immunologically cross-reactive HisDCase from Lactobacillus buchneri (81 residues) and Clostridium perfringens (86 residues) were then determined to be a and b, respectively.

Although these sequences differ substantially near the NH\(_2\)-terminal ends, there is striking homology near the COOH termini and also near the NH\(_2\) terminus of the two \(\alpha\) chains (pyruvyl-Phe-X-Gly-Val-, where X is Ser or Cys). If the four known pyruvyl-dependent HisDCases arise from inactive proenzymes by the mechanism previously demonstrated for the HisDCase from Lactobacillus 30a (Recei, P. A., Huynh, Q. K., and Snell, E. E. (1984) Annu. Rev. Biochem. 53, 357–387), then each of these proenzymes has the sequence -Thr-Ala-Ser-Ser-Phe- at the activation site (2). The complete sequences of the \(\alpha\), \(\beta\), and \(\gamma\) chains of other pyruvoyl-dependent HisDCases would be of interest. We describe here the purification to homogeneity of HisDCase from Lactobacillus buchneri and some of its properties, the complete sequence of the \(\beta\) chain of HisDCase from L. buchneri and from Clostridium perfringens, and an extended NH\(_2\)-terminal sequence of the \(\alpha\) chains from these same two enzymes. Together with published data (3–5) these results show that although major differences in sequence occur elsewhere, all four of the known HisDCases show strikingly similar sequences around the active pyruvyl residue and hence around the activation site of their putative proenzymes.

**EXPERIMENTAL PROCEDURES AND RESULTS**

HisDCase from L. buchneri is cross-reactive with antibodies to HisDCase from Lactobacillus 30a, but the two enzymes are not identical (6). Immunoprecipitates of the L. buchneri enzyme were used previously to establish that this enzyme like that from Lactobacillus 30a has an \((\alpha\beta)_6\) structure, contains a pyruvoyl group on one of the subunits, and is formed from an inactive pyruvoyl-dependent proenzyme (6). We have now purified this enzyme to homogeneity by a more conventional 4-step procedure (Table II, Miniprint). It has an \((\alpha\beta)_6\) structure and closely resembles the Lactobacillus 30a enzyme in its catalytic parameters (Table III, Miniprint). This preparation of the L. buchneri enzyme and a homogeneous sample of the HisDCase from C. perfringens prepared as described previously (6) were then used to prepare the separate \(\alpha\) and \(\beta\) subunits from each enzyme for comparative sequence studies.

The complete amino acid sequences of the \(\beta\) chains of HisDCases from C. perfringens and L. buchneri are shown in Fig. 1, A and B, respectively. The C. perfringens \(\beta\) chain contains 86 residues (M\(_r\) = 8317); the L. buchneri \(\beta\) chain contains 81 residues (M\(_r\) = 8810). These values agree with

Four pyruvyl-dependent HisDCases\(^4\) are now known (1). All of them have an \((\alpha\beta)_6\) subunit structure, with the essential pyruvoyl group blocked by a serine residue at the cleavage site (1, 2). Immunopotentiates of the L. buchneri enzyme were used previously to establish that this enzyme like that from Lactobacillus 30a has an \((\alpha\beta)_6\) structure, contains a pyruvoyl group on one of the subunits, and is formed from an inactive pyruvoyl-dependent proenzyme by similar mechanisms, and have similar catalytic mechanisms.

\(^{4}\) Portions of this paper (including "Experimental Procedures," "Results," Fig. 3, and Tables II–XVI) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9350 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-5576, cite the authors, and include a check or money order for $8.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
FIG. 1. The complete amino acid sequences of the \( \beta \) chains of histidine decarboxylases from \( C. \) perfringens (A) and \( L. \) buchneri (B). Full arrows (---) indicate identification by HPLC of phenylthiohydantoins following automated sequencing. Half-arrows (--) indicate identification by manual sequencing. Reverse arrows (--) indicate identification by carboxypeptidase digestion. Data printed above the sequences are from steps performed on the intact proteins and those below the sequences are from steps performed on the digestion products. Peptides are designated: \( T \), tryptic; \( C \), chymotryptic; and \( CB \), cyanogen bromide.

Those previously estimated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6). The amino acid compositions determined from the sequences are in good agreement with those obtained by amino acid analyses (Table IV, Miniprint).

One Met-Thr sequence occurs in the \( L. \) buchneri \( \beta \) chain at positions 77-78. Such a sequence has previously been reported to be resistant to CNBr (7) and was cleaved in the \( L. \) buchneri \( \beta \) chain only to the extent of about 15% (Table XIV, Miniprint). There were, in contrast, some chymotryptic and subtilisin-like cleavages during digestion of the \( \beta \) chains of both enzymes with trypsin (peptide T3b of the \( L. \) buchneri HisDCase and peptide T1 of the \( C. \) perfringens (HisDCase, Fig. 1). Such autolytic development of chymotryptic and subtilisin activities during tryptic digestion has been reported previously (8-10). The other unexpected result was our failure to find the NH\(_2\)-terminal peptide (Thr-Leu-Ser) of the \( C. \) perfringens \( \beta \) chain during purification of the tryptic peptides by HPLC.
This peptide sequence, however, was determined by analysis of the NH2-terminal sequence on the intact β chain (Table IX) and confirmed by the sequences of chymotryptic peptide C1 and cyanogen bromide peptide CB1 (Fig. 1A). Only single-residue overlaps of isolated peptides are shown in Fig. 1 for the β chain of the C. perfringens enzyme at residues 27–28 and 47–48 and for that of the L. buchneri enzyme at residues 20–21 and 42–43. These positional assignments are further supported by the results of automated sequencing from the NH2 terminus of the intact β chains (Tables IX and XI, Miniprint) and by the amino acid compositions of cyanogen bromide peptides, CB1 and CB2 (Table X and XIV, Miniprint).

A comparison of the sequences of the β chains from four sources is shown in Fig. 2, which also compares the NH2-terminal sequences of the α chains from these same four sources. The L. buchneri β chain shows high homology (96%) with the β chain of the Lactobacillus 30α enzyme, but there is much less homology among the β chains of HisDCases from C. perfringens, Lactobacillus 30α and Micrococcus sp. n. (Table I). These results are consistent with the findings that rabbit antibodies prepared against HisDCase from Lactobacillus 30α cross-react with HisDCase from L. buchneri but not with that from C. perfringens (6). Only 23% of the β chain residues are identical in all four enzymes, and most of these residues are concentrated near the COOH-terminal portion of the β chain (Fig. 2). Similarly, residues near the essential pyruvyl residue at the NH2 terminus of the α chain are more highly conserved than those further removed (Fig. 2). In proHisDCase from Lactobacillus 30α the carboxyl-terminal serine (Ser) of the β chain and the serine precursor (Ser) of the α chain pyruvyl residue are linked in the proenzyme sequence, 'Thr-Ala-Ser-Ser-Phe', that overlaps the COOH terminus of the β chain ('Thr-Ala-Ser') and the NH2 terminus (Pyr-Phe-) of the α chain (3, 11). Putative proenzymes for each of the four HisDCases would have this same sequence, with highly conserved sequences on either side (Fig. 2). Although such proenzymes have been detected so far only in Lactobacillus 30α (12) and L. buchneri (6), these considerations, together with the common properties described in Table III, indicate that the four enzymes have evolved from a common ancestral protein, that they are formed from inactive pyruvate-free proenzymes by a mechanism similar to that described for the Lactobacillus 30α enzyme (2), and that active sites in the mature enzyme and activation sites in the postulated proenzymes are conformationally similar with probably similar catalytic residues.

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Sequences of β Chains from Histidine Decarboxylases

Comparison of β subunits from histidine decarboxylases from various sources revealed a high degree of homology.

The β subunit of human histidine decarboxylase was found to have 96% identity with the corresponding region of the β subunit of E. coli histidine decarboxylase.

The β subunit of M. tuberculosis histidine decarboxylase was found to have 94% identity with the corresponding region of the β subunit of E. coli histidine decarboxylase.

The sequence of the β subunit of S. typhimurium histidine decarboxylase was also determined and compared with the sequences of the β subunits from other species.

In conclusion, the high degree of homology observed among the β subunits of histidine decarboxylases from different sources suggests a conserved evolutionary origin and functional role for this subunit.
TABLE III. Amino Acid Composition of the Tryptic Peptides from the α Chain (100 μg) of Histidine Decarboxylase from L. lactis\(^*\)

| Amino Acid | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 |
|------------|----|----|----|----|----|----|----|----|
| Tyr        | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) |
| His        | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) |
| Val        | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) |
| Ser        | 1.2(1) | 1.2(2) | 0.8(1) | 0.6(1) | 0.6(1) | 0.6(1) |
| Ala        | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Ile        | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Met        | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Leu        | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Val        | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Phe        | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Total      | 10  | 10  | 10  | 10  | 10  | 10  |
| Yield (μg) | 11 | 11 | 11 | 11 | 11 | 11 |

\(\alpha\) See corresponding footnotes for Table III.

TABLE IV. Amino Acid Composition of the Cleavable Peptides from the α Chain (100 μg) of Histidine Decarboxylase from L. lactis\(^*\)

| Amino Acids | C3 | C4 | C5 | C6 | C7 | C8 |
|-------------|----|----|----|----|----|----|
| Tyr         | 1.2(1) | 1.2(1) | 1.2(1) | 1.2(1) | 1.2(1) | 1.2(1) |
| His         | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) |
| Val         | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) |
| Ser         | 1.2(1) | 1.2(2) | 0.8(1) | 0.6(1) | 0.6(1) | 0.6(1) |
| Ala         | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Ile         | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Met         | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Leu         | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Val         | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Phe         | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Total       | 10  | 10  | 10  | 10  | 10  | 10  |
| Yield (μg)  | 11 | 11 | 11 | 11 | 11 | 11 |

\(\alpha\) See corresponding footnotes for Table IV.

TABLE V. Automatic Sequence Analysis of the α Chain from L. lactis Histidine Decarboxylase (100 μg)

| Cycle Number | Residue | Yield (μg) | Cycle Number | Residue | Yield (μg) |
|--------------|---------|------------|--------------|---------|------------|
| 1            | Phe     | 3.0        | 10            | Asp     | 10.7       |
| 2            | Phe     | 10.9       | 11            | Ser     | 0.6        |
| 3            | Lys     | 5.2        | 12            | Ser     | 6.2        |
| 4            | Gly     | 5.8        | 13            | Ala     | 4.4        |
| 5            | Val     | 11.0       | 14            | Asp     | 4.7        |
| 6            | Ala     | 8.0        | 15            | Asp     | 2.3        |
| 7            | Gly     | 5.3        | 16            | Glu     | 1.0        |
| 8            | Glu     | 6.7        | 17            | Glu     | 2.9        |
| 9            | Trp     | 6.3        | 18            | Leu     | 1.3        |
| 10           | Trp     | 6.0        | 19            | Leu     | 1.3        |
| 11           | Val     | 4.4        | 20            | Trp     | 2.3        |
| 12           | Val     | 4.1        | 21            | Ser     | 1.1        |
| 13           | Ala     | 4.1        | 22            | Glu     | 1.1        |
| 14           | Ala     | 6.6        | 23            | Leu     | 1.3        |
| 15           | Ala     | 6.6        | 24            | Ala     | 1.4        |
| 16           | Thr     | 2.1        | 25            | Glu     | 0.8        |
| 17           | Ser     | 0.8        | 26            | Glu     | 0.8        |

\(\alpha\) See corresponding footnotes for Table V.

\(\beta\) Determined as alanine after reductive amination.

\(\gamma\) See corresponding footnotes for Table IV.

\(\delta\) See corresponding footnotes for Table IV.

\(\epsilon\) Determined as homoserine plus homoserine and its lactone.