Pyruvoyl-dependent Histidine Decarboxylases

MECHANISM OF CLEAVAGE OF THE PROENZYME FROM LACTOBACILLUS BUCHNERI

Paul A. Recsei and Esmond E. Snell
From the Departments of Microbiology and Chemistry, The University of Texas, Austin, Texas 78712

When Lactobacillus buchneri was grown in the presence of [hydroxy-18O]serine and pyridoxamine, no 18O was found in its histidine decarboxylase (HisDCase). However, when pyridoxamine was omitted from the growth medium, the labeled serine was incorporated into the HisDCase without dilution. Internal serine residues of the enzyme contained 18O only in their hydroxyl group, while the COOH-terminal serine of the β chain of HisDCase contained equal amounts of 18O in both its hydroxyl and carboxyl group. This enzyme, like the HisDCase from Lactobacillus 30a (Recsei, P. A., Huynh, Q. K., and Snell, E. E. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 973–977), therefore, arises by nonhydrolytic serinolysis of its proenzyme. This result, together with comparative sequence data (Huynh, Q. K., and Snell, E. E. (1985) J. Biol. Chem. 260, 2798–2803), makes it highly probable that all of the pyruvoyl-dependent HisDcases arise by a similar mechanism from inactive proenzymes.

A so far unique instance of the nonhydrolytic cleavage of a peptide chain in vivo is provided by activation of prohistidyl decarboxylase in Lactobacillus 30a (1). This reaction proceeds by a single turnover, intramolecular serinolysis, in which a two daughter subunits, α and β, in the active decarboxylase (HisDCase) according to Equation 1 (2).

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\begin{align*}
\text{H}_2\text{O} + \text{NH}_3 &\rightarrow \beta \text{ subunit} + \alpha \text{ subunit} \\
\text{Thr-Ala-Ser-Ser-Phe-} &\rightarrow \text{Thr-Ala-SerOH} + \text{Prv-Phe-} \\
\end{align*}
\]

(1)

Oxygen from H218O is not incorporated into the newly generated carboxyl terminus of the β chain; instead, this oxygen is supplied by the hydroxy group of the serine residue (Ser in Equation 1) that becomes the pyruvate residue (Prv) of the active enzyme (2).

That this mechanism, initially demonstrated for a mutant proenzyme with a slowed activation rate, also pertains for activation of wild-type proHisDCase under growth conditions was demonstrated by supplying [hydroxy-18O]serine to the growing cultures of Lactobacillus 30a and showing that while control serine residues from the α chain of the subsequently isolated HisDCase contained 18O only in their hydroxyl groups, the carboxyl-terminal serine residue from the β chain contained equal amounts of 18O in both the hydroxyl and carboxyl groups (2).

Pyruvoyl-dependent HisDcases from three other bacterial species are now known (1). Although proHisDCase has been detected in only one of these, Lactobacillus buchneri (1), the finding (3) that HisDCases from all three organisms have the same sequence at the β chain COOH terminus and the same sequence at the α chain NH2 terminus as that shown for the Lactobacillus 30a enzyme in Equation 1 implies that all four enzymes arise from inactive proenzymes that share a common activation sequence and that activation proceeds by the same mechanism in each case. To test this implication, we grew L. buchneri with [hydroxy-18O]serine under the same conditions used previously for Lactobacillus 30a and found to our surprise that none of the serine residues from the isolated HisDCase contained 18O. However, as described below, when pyridoxamine was omitted from the growth medium to minimize serine degradation and synthesis, the same transfer of 18O observed in Lactobacillus 30a was observed in L. buchneri.

EXPERIMENTAL PROCEDURES

Fractionation and Immobilization of Rabbit Antibodies to Histidine Decarboxylase—Rabbit antiserum (100 ml) to histidine decarboxylase from Lactobacillus 30a was prepared as described previously (4). The precipitate that formed upon addition of sodium sulfate at 25 °C to 18% concentration was collected by centrifugation, dissolved in 50 ml of 0.15 M NaCl, and reprecipitated by addition of sodium sulfate (final concentration, 10%). The precipitate was collected by centrifugation, dissolved in 50 ml of 17 mM potassium phosphate, pH 6.3 (Buffer A), and dialyzed against this buffer for 24 h at 0 °C. The solution was then clarified by centrifugation and applied to a column of DE-52 (30 ml) equilibrated with Buffer A (5). Protein-containing fractions obtained by elution of the column with Buffer A were pooled, and the precipitate that formed upon addition of ammonium sulfate at 0 °C to 50% of saturation was collected by centrifugation, dissolved in 15 ml of 0.15 M NaCl, and dialyzed against 0.15 M NaCl, 1 mM sodium borate, pH 8.4 (Buffer B) for 24 h at 0 °C. The yield of immunoglobulin, determined spectrophotometrically at 280 nm using the E1% = 15, was 490 mg. Agarose (30 ml, Sepharose 4B) suspended in 120 ml of 1 M sodium carbonate was activated with 5 g of cyanogen bromide in 5 ml of acetonitrile for 2 min at 0 °C (4), washed with 400 ml of ice-cold water, and gently stirred with 450 mg of immunoglobulin in 40 ml of buffer B. After 14 h at 4 °C less than 1% of the protein remained in solution. The gel was washed with 1 liter each of Buffer B and 0.1 M ammonium acetate, pH 4.8, and stored in Buffer B.

Purification of HisDCase from L. buchneri— Cultures (3 liters) were grown to stationary phase on crude medium (6) or on the defined medium of Guirard and Snell (7) modified to contain l-histidine (5 g/liter) and L-[hydroxy-18O]serine (15 mg/liter). In some cases the defined medium was further modified by omission of pyridoxamine and addition of D-glutamic acid (30 mg/liter). The cells were har-
vested by centrifugation, washed with cold water, and acetone dried. Acetone-dried cells (2 g) were suspended in 20 ml of 0.2 M ammonium acetate, pH 4.8, and subjected to sonication oscillation for a total of 9 min using an Ultrasonic model W-375 sonicator. Insoluble material was removed by centrifugation. The pH of the supernatant solution was adjusted to neutrality by addition of 5 N NaOH, and the solution was stirred for 5 min to elute the bound enzyme. The supernatant was collected, neutralized by addition of 1.5 ml of 1 M potassium phosphate, pH 7.6, reduced in volume by ultrafiltration using an Amicon YM-10 membrane, dialyzed against water for 24 h, and lyophilized. Purity of preparations was confirmed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (8). The yield of purified enzyme was around 5 mg.

Isolation and Enzymatic Digestion of the α and β Chains of HisDCase—Purified HisDCase was incubated in 3 ml of 5 M guanidinium chloride, 0.03 M sodium phosphate, pH 7.6, for 2 h at 37 °C. The solution was then dialyzed against 0.03 M sodium phosphate, 1 mM β-mercaptoethanol, pH 7.6, for 2 days at 25 °C and applied to a column of DE-52 (2 ml) equilibrated with 0.03 M sodium phosphate, pH 7.6, β and α chains were eluted in that order with this buffer containing 0.1 or 0.4 M NaCl, respectively. Protein-containing fractions were dialyzed against distilled water and lyophilized. Purity of preparations was determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (8). The β chain (0.4 mg) was incubated in 0.1 ml of 0.2 M pyridine, acetic acid, pH 6.0, for 30 min at 30 °C and then digested with carboxypeptidase Y (2 μg) for varying periods of time. Digestion in this buffer containing 90 atom % ¹³C was carried out for 30 min at 30 °C. The solution was heated at 100 °C for 4 min to stop the reaction and lyophilized. The residue was suspended in 90% ethanol (0.4 ml), and the soluble portion was subjected to amino acid analysis and gas chromatography/mass spectrometry. Conditions for digestion of the α and β chains (0.4 mg each) of HisDCase isolated from cells of Lactobacillus 30α grown in the presence of L-[hydroxyl-³³C]serine have been described (2); the same procedures were used with the enzyme from L. buchneri.

Other Methods—Synthesis of L-[hydroxyl-³³C]serine and procedures used for trimethylsilylation and gas chromatography/mass spectrometry have been described (2). Histidine decarboxylase activity was determined manometrically (9).

Materials—H₂¹³CO (90 atom % ³³C) was obtained from Prochim, N-tosyl-l-phenylalanine chloromethyl ketone-treated trypsin from Millipore, DE-52 from Whatman, Sepharose 4B from Pharmacia, and carboxypeptidase Y from Pierce.

RESULTS

Identification of the COOH-terminal Amino Acid of the β Chain—Digestion of the β chain of histidine decarboxylase from L. buchneri with carboxypeptidase resulted in the rapid release of nearly 1 serine residue/mol of β chain (Fig. 1). Alanine, threonine, and methionine were released at slower rates. When carboxypeptidase digestion was carried out in ³³C-enriched water, no ³³C label was present in the serine carboxyl group while the other amino acids released contained the theoretical amount of ³³C in their carboxyl groups (Table I). These results confirm that the COOH-terminal residue of the β chain and that the serine residues released by carboxypeptidase digestion were derived solely from the COOH terminus of the chain. They agree fully with the known carboxyl-terminal sequence (M-T-T-A-S) for the β chain of this protein (3).

Analysis of Histidine Decarboxylase from Cells Grown in the Presence of L-[hydroxyl-³³C]Serine—When histidine decarboxylase was isolated from L. buchneri grown on defined medium containing 33 atom % L-[hydroxyl-³³C]serine, essentially no label was present in the serine residues of either the α or the β chain (Fig. 2A). When the defined medium was modified by the addition of all d- and L-amino acids required for cell growth and omission of pyridoxamine to minimize degradation of added ³³C-serine and/or synthesis of unlabeled L-serine by the cells, L-[hydroxyl-³³C]serine was incorporated into protein with essentially no dilution of label (Fig. 2B). Serine residues from the α chain contained 31 atom % ³³C in the hydroxyl group and essentially no ³³C in the carboxyl group. The COOH-terminal serine of the β chain contained 33 atom % ³³C in the hydroxyl group, and in addition 31% of the carboxyl groups of this residue contained ¹³C (Fig. 2C and Table II). No ¹³C was present in the other amino acid residues of the enzyme. This result was also observed with HisDCase from Lactobacillus 30α and demonstrates that the hydroxyl oxygen of a serine residue is incorporated into the newly formed carboxyl group at the COOH terminus of the β chain during proenzyme activation in both organisms.

DISCUSSION

Lactobacillus 30α is a nutritionally demanding organism; it requires both vitamin B-6 and each of the amino acids, including serine, for growth (7). Its incorporation of added ³³C-serine into HisDCase, as reported earlier (2), is, there-
FIG. 2. Mass spectra from m/z 200 to 230 of the trimethylsilyl derivative of serine derived from L. buchneri HisDCase. A, serine residues released from the β chain by digestion with carboxypeptidase Y in H218O. Unlabeled standard and serine residues from the α and β chains isolated from cells grown on defined medium containing both L-[hydroxyl-18O]serine and vitamin B-6 (pyridoxamine) showed this same spectrum. B, serine residues from the α chain or C, the COOH-terminal serine residue from the β chain, both from HisDCase of cells grown on defined medium containing L-[hydroxyl-18O]serine but modified by omission of vitamin B-6. The spectral peak at m/z 204 represents Me3Si serine (unlabeled) that has lost the fragment containing the carboxyl group of serine; that at 218 is the fragment that lacks the \( \text{CH}_3\text{OSiMe}_3 \) group (see references in Ref. 2).

| Source of serine | Hydroxyl content | Carboxyl content |
|------------------|------------------|------------------|
| α chain          |                 |                  |
| COOH-terminal    | 33               | <1               |
| of the β chain   |                  |                  |

These values were determined from the intensities of the ions at m/z \( M - R \) and \( M - 117 \) (2); see also Fig 2.

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by nonhydrolytic serinolysis. This reaction results in transfer of the hydroxyl oxygen of the serine precursor of the pyruvoyl group into the newly formed COOH terminus and probably proceeds via an ester intermediate (2). The present results, together with the finding (3) that each of the pyruvyl-dependent HisDCases known share a common amino acid sequence at the presumed activation site, indicate that this mechanism for proenzyme cleavage and formation of the essential pyruvoyl residue is general for this group of enzymes. Whether it also accounts for formation of the essential pyruvoyl group of other enzymes that contain this residue is not yet known.