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Biochemistry

Amine cations promote concurrent conversion of prohistidine decarboxylase from *Lactobacillus* 30a to active enzyme and a modified proenzyme

(α,β-elimination/nonhydrolytic serinolysis/protein processing/pyruvoyl enzymes/histidine and sodium binding)

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ABSTRACT **Activation of prohistidine decarboxylase (π_6) from *Lactobacillus* 30a proceeds by an intramolecular, pH- and monovalent cation-dependent reaction in which its constituent π chains are cleaved nonhydrolytically between Ser-81 and Ser-82 with loss of NH_3 and conversion of Ser-82 to the pyruvoyl residue of active histidine decarboxylase $(\alpha\beta)_6$. Amines with pKa values more than 7.0 substitute for K^+ or NH_4^+ in the activation of prohistidine decarboxylase, but they also catalyze its inactivation in a competing reaction, $\pi_6 \rightarrow \pi'_6$. Sequence analysis of the appropriate tryptic peptide from amine-inactivated prohistidine decarboxylase established that inactivation results from conversion of Ser-82 of the π chain to an aminoacrylate residue. The inactivated proenzyme (π'_6) does not form histidine decarboxylase; this fact eliminates one of two postulated mechanisms of activation and, thus, favors activation by β-elimination of the acyl group of an intermediate ester formed between Ser-81 and Ser-82. L-Histidine is bound by the proenzyme ($K_d = 1.7 \times 10^{-4}$ M) and is an effective activator; one binding site is present per π subunit. K^+, NH_4^+, and Na^+ competitively inhibit (K_i values = $2.8–4.4 \times 10^{-3}$ M) activation by histidine. The data suggest the presence of two classes of monovalent cation binding sites on prohistidine decarboxylase: one (near Ser-82) is readily saturable and one is unsaturable even by 2.4 M K^+.**

Histidine decarboxylase (HisDCase) from *Lactobacillus* 30a is representative of a number of amino acid decarboxylases now known to contain a catalytically essential pyruvoyl group (1). In the three cases so far studied (2–4), this pyruvate residue arises from a serine residue of a pyruvate-free proenzyme (proHisDCase) and is formed coincident with cleavage of the precursor (π chain) subunit of the proenzyme into the α and β chain subunits of the active enzyme. In each of two cases studied (3, 5), this conversion proceeds by an unusual process, termed a nonhydrolytic serinolysis, in which chain cleavage between Ser-81 and Ser-82 of the π subunit is accompanied by the quantitative transfer of the side-chain oxygen of Ser-82 to the carboxyl residue of Ser-81 to form the COOH terminus of the β subunit, while Ser-82 becomes the pyruvate residue blocking the NH_2 terminus of the newly formed α subunit of HisDCase. In the presence of monovalent cations (K^+ and NH_4^+ are most active), which are required for the reaction, the conversion of proHisDCase to HisDCase is essentially complete (6).

In this paper we describe the specificity of this requirement for monovalent cations. A variety of organic amines, including histidine, was found to replace K^+ or NH_4^+; however, they also catalyze an interesting competing reaction that leads to inactivation of proHisDCase by conversion of Ser-82 to an α-aminoacrylate residue. The findings eliminate one of the postulated routes from proHisDCase to HisDCase.

MATERIALS AND METHODS

Materials. Sephadex G-50 (superfine), Sephacryl S-200 (superfine), cyanogen bromide, and L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin were obtained from Sigma; carboxypeptidase Y and amino acid sequencing reagents ("sequanal grade"), from Pierce; Dowex 1-X8 anion exchange resin, from Bio-Rad; nitrocellulose sheets (0.45-μm pore size), from Schleicher & Schuell; and L-[*carboxyl*-^{14}C]histidine, from New England Nuclear. Other chemicals were of reagent grade from standard sources.

Purification of HisDCase. HisDCase was isolated from *Lactobacillus* 30a mutant 3 as described by Chang and Snell (7) for HisDCase, with omission of the acetone fractionation and crystallization steps.

Measurement of HisDCase Activity. Enzyme activity was measured by release of $^{14}CO_2$ from carboxyl-labeled L-histidine (8, 9) in 0.2 M ammonium acetate (pH 4.8). Specific activity represents μmol of CO_2 formed per mg of enzyme per min.

Protein Determinations. proHisDCase/HisDCase concentrations were determined either by their absorbance at 280 nm (7) or by the method of Lowry *et al.* (10) with bovine serum albumin or proHisDCase as a standard.

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Electrophoresis was performed in the presence of 1% $NaDodSO_4$ on slab gels (separating gels, 10% acrylamide/0.27% methylenebisacrylamide; stacking gels, 4% acrylamide/0.1% methylene bisacrylamide) as described by Laemmli (11). Protein bands were visualized with Coomassie blue by the method of Fairbanks *et al.* (12). For preparative purposes, the desired bands were excised from the gel, and the gel slices were eluted electrophoretically with the use of a Savant preparative acrylamide gel cell. Protein eluates from the gels were dialyzed against deionized water and lyophilized. The protein was then redissolved in 2 ml of 6 M urea/0.05 M NH_4CO_3 and passed through a Dowex 1 column (15 cm × 0.5 cm) equilibrated and eluted with the same buffer (13). Fractions containing the protein were pooled, and the urea was removed by dialysis against deionized water.

High Performance Liquid Chromatography. HPLC was performed on a Tracor instrument equipped with a 950 A chromatographic pump, a 970 A variable wavelength detector, and a 980 A solvent programmer. A reverse-phase Synchropak RP-P-300 semipreparative column (25 cm × 1 cm, SynChrome, Linden, IN) was used for all separations.

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Abbreviations: proHisDCase, prohistidine decarboxylase; HisDCase, histidine decarboxylase; DABTH, dimethylaminobenzene thiohydantoin; $N(EtOH)_3$, triethanolamine.

Peptide Sequencing. The 4-*N*,*N*-dimethylaminobenzene 4'-isothiocyanate/phenylisothiocyanate double-coupling method (14) was used for manual sequencing of peptides. Dimethylaminobenzene thiohydantoin (DABTH) derivatives of amino acids were identified on polyamide sheets.

RESULTS

Activation and Inactivation of proHisDCase by Triethanolamine [N(EtOH)$_3$]. Like K^+ or NH_4^+ ions (6), $N(EtOH)_3$ catalyzed the activation of proHisDCase, a reaction evident from the cleavage of the π chains of the proenzyme to the α and β chains of HisDCase (Fig. 1*A*) and the concomitant appearance of HisDCase activity (Fig. 1*B*, curve 1). Pro-HisDCase activation by $N(EtOH)_3$ resembled the K^+-catalyzed process in many respects: the pH optimum of activation was approximately 7.5, there was a half-order relationship between the rate of activation and the amine concentration, and saturation of the proenzyme by amine was not observed. In contrast to the K^+-catalyzed reaction, however, activation by $N(EtOH)_3$ did not proceed to completion because of a competing inactivation reaction that is recognized by (*i*) a progressive decrease with time in the fraction of proHisDCase that can be activated to HisDCase in the presence of K^+ (Fig. 1*B*, curve 2) and (*ii*) the cessation of π chain cleavage after 24 hr of exposure to the amine (Fig. 1*C*). The inactive product (product I) that remained after the cleavage reaction stopped consists of subunits indistinguishable in molecular weight from the π chain of proHDC (Fig. 1*A*). Product I was not cleaved to the α and β chains of HisDCase by K^+, even after exhaustive dialysis against deionized water.

$N(EtOH)_3$-catalyzed inactivation of proHisDCase did not occur below pH 6.5. Above this pH, the rate of inactivation increased linearly with increasing pH. The pH optimum of inactivation could not be established because, at pH values around 9.0, it was difficult to distinguish between the effects of the amine and other factors; the proenzyme was inactivated at this pH in K^+-based buffers as well. The inactivation of proHisDCase by $N(EtOH)_3$, like the activation reaction, displayed a half-order relationship between rate of inactivation and the amine concentration. No saturation of the proenzyme by amine was evident. K^+, NH_4^+, or Na^+ (25 mM) protected the proenzyme completely against amine-catalyzed inactivation.

Isolation and Sequence Analysis of a Peptide from Product I Corresponding to the Cleavage Site of proHisDCase. To determine whether product I formation results from a change at the cleavage site of proHisDCase, a peptide corresponding to this site was isolated from amine-treated proenzyme (product I). Isolation of the peptide was greatly facilitated by knowledge of the primary structure of the proenzyme (15, 16). Since a methionyl-threonyl bond is located four amino acids upstream from the Ser-81/Ser-82 cleavage site of proHisDCase, the proenzyme (25 mg), first treated with 0.1 M $N(EtOH)_3$ (pH 8.0) at 37°C for 24 hr, was subjected to cyanogen bromide cleavage as described by Gross and Witkop (17). The cleavage products were separated by gel filtration (Fig. 2*A*), and the fragment containing the cleavage site was identified by its NH_2-terminal sequence (T-T-A) and was further purified by HPLC (Fig. 2*B*). A tryptic digestion of this purified fragment (with T-T-A at its NH_2 terminus) was

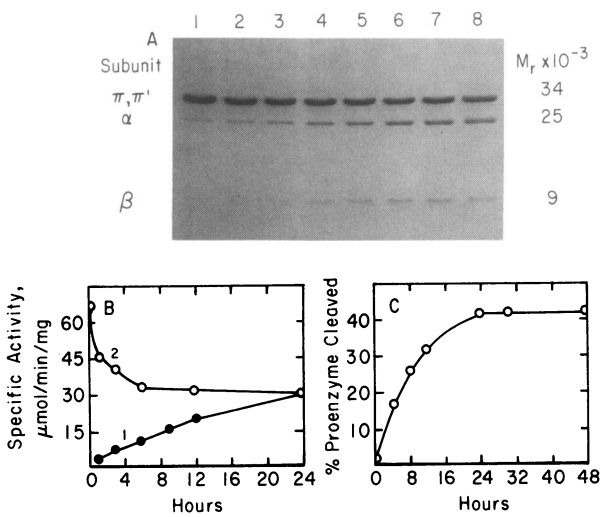

FIG. 1. The activation and inactivation of proHisDCase by $N(EtOH)_3$. (*A*) Cleavage of the proHisDCase π chain to the α and β chains of HisDCase and conversion of HisDCase to product I (see text) were followed by the $NaDodSO_4$/polyacrylamide gel electrophoresis of samples taken after 0, 2, 4, 8, 12, 24, 30, and 48 hr of incubation (lanes 1–8, respectively) of the proenzyme at 37°C in 0.1 M $N(EtOH)_3$ (pH 8.0). (*B*) The rate of activation (curve 1) of proHisDCase under the conditions described above was determined by measuring the appearance of HisDCase activity with time. The rate of inactivation (curve 2) of the proenzyme was measured in aliquots removed from the incubation mixture at various time intervals and then exposed to 1 M K^+ (pH 7.6) at 37°C for 24 hr prior to HisDCase assay. (*C*) The rate and extent of cleavage of proHisDCase were estimated by measuring the decrease in optical density of the π,π' band on the gel in *A* with a Bio-Rad 620 video densitometer.

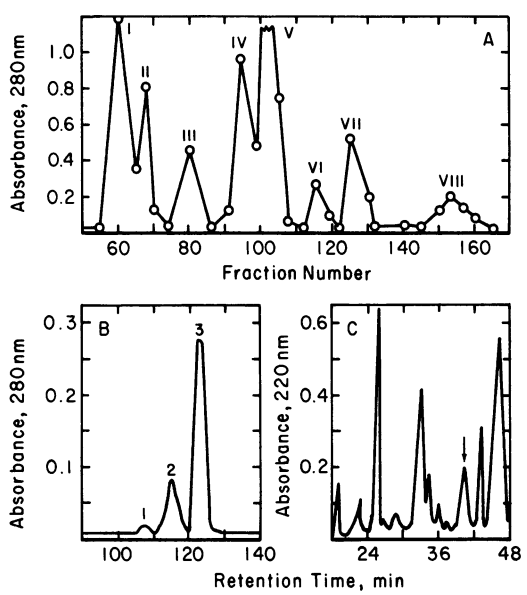

FIG. 2. Purification of a peptide from product I corresponding to the cleavage site of proHisDCase. (*A*) Sephadex G-50 chromatography of the cyanogen bromide digest of proHisDCase treated with $N(EtOH)_3$. After application of the sample (in 2.5 ml of 30% acetic acid) the column (150 cm × 1.5 cm) was eluted at a flow rate of 6 ml/hr with 30% aqueous acetic acid, and fractions (1.5 ml) were collected. Fractions 62–70 ("peak II") contained the peptide of interest and were pooled and lyophilized. (*B*) Further purification of peak II by reverse-phase HPLC. The sample (in 500 μl of 1% ammonium bicarbonate) was injected, and a gradient was run from 0.1% aqueous trifluoroacetic acid to 50% CH_3CN in 0.1% aqueous trifluoroacetic acid (flow rate, 1 ml/min; gradient increase, 0.83%/min) The second peak ("peak II-2") was the desired peptide. (*C*) Reverse-phase HPLC of the tryptic digest of peak II-2. Fractions containing the peak II-2 peptide (0.55 mg) were lyophilized, redissolved in 1% ammonium bicarbonate (200 μl) and then digested with TPCK-treated trypsin (6 hr at 37°C) with an enzyme to substrate ratio of 1:50. The digest was then separated by HPLC on a reverse-phase column under the same elution conditions as those described in *B* except that the final concentration of CH_3CN was 80% and the rate of gradient increase was 1.25%/min. The peptide from product I corresponding to the cleavage site of proHisDCase is indicated by an arrow.

then performed (see the legend to Fig. 2C), since in the intact proenzyme an arginyl-glycyl bond present seven amino acids downstream from Ser-82 should provide a tryptic cleavage site. The resulting tryptic peptides were separated by HPLC (Fig. 2C), and the peptide of interest once again was located by NH_2-terminal amino acid sequencing. A second HPLC separation (performed as described in Fig. 2B) removed a contaminating peptide that copurified with the desired peptide. The total yield of pure peptide (as determined by amino acid analysis) was approximately 15 nmol, well below the theoretical yield of 400 nmol. The most apparent reason for this low yield is the known resistance of methionyl-threonyl bonds to cyanogen bromide cleavage (18).

As a control, the corresponding tryptic peptide containing the cleavage site of untreated proHisDCase was isolated by the same procedure. The sequence of this peptide (Fig. 3, line A) corresponds to that of residues 78–89 of the proHisDCase π chain. Sequencing of the peptide from $N(EtOH)_3$-treated proenzyme yielded the same sequence (Fig. 3, line B) except for an altered residue at position 82 that did not yield the DABTH derivative of serine but did yield derivatives of three degradation products of serine [DABTH-dehydroalanine, DABTH-polymerized dehydroalanine, and DABTH-hydrated, polymerized dehydroalanine (19)]. This finding suggests that product I formation results from conversion of Ser-82 of proHisDCase to a dehydroalanyl residue. The presence of dehydroalanine at this position was confirmed by reduction of the modified peptide with $NaBH_4$ (see the legend to Table 1). Subsequent sequencing showed the presence of alanine, the expected reduction product of dehydroalanine, at position 82 (Fig. 3, line C). The amino acid composition of the reduced peptide (Table 1) is in complete agreement with this sequence.

Activation and Inactivation of proHisDCase by Other Amines. Other amines—primary, secondary, tertiary, and quaternary—acted in the same way as $N(EtOH)_3$ (Table 2). The rates of activation catalyzed by the amines were in the same range as that catalyzed by NH_4^+. In view of the high pK_a values of the amines tested, their activating effect is likely to be mediated by their cations. In contrast, the number and nature of the groups linked to the amine nitrogen were important in determining the rate of inactivation: secondary, tertiary, and quaternary amines catalyzed much higher rates of inactivation than did primary amines, whereas the smaller ammonium ions did not catalyze the reaction at all.

To determine whether the same inactivation reaction was catalyzed by all of the amines listed in Table 2, the modified π chain from each reaction mixture (0.1 M amine at pH 8.0 for 24 hr at 37°C) was separated from activation products by preparative polyacrylamide electrophoresis. The dehydroalanine content of these modified π chains (10–15 nmol) was then assayed by the method of Patchornik and Sokolovsky (20). This procedure converts dehydroalanyl residues to pyruvoyl groups and liberates the latter from the protein as pyruvic acid by mild acid hydrolysis. The pyruvic acid formed was assayed with 2,4-dinitrophenylhydrazine as described by Koepsell and Sharpe (21). The spectral properties of the dinitrophenylhydrazones formed were identical in each case to those of pyruvic acid dinitrophenylhydrazone. In each case (Table 2), an approximate 1:1 stoichiometry of dehydroalanine (assayed as pyruvic acid) to protein was obtained. Untreated proenzyme, examined as a control, did not contain dehydroalanine.

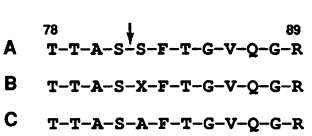

FIG. 3. Amino acid sequence of the tryptic peptide containing the cleavage site of proHisDCase (line A), the corresponding tryptic peptide from $N(EtOH)_3$-treated proHisDCase (product I; line B), and the latter peptide reduced with $NaBH_4$ prior to sequencing (line C). The peptide bond cleaved during proHisDCase activation is indicated with an arrow.

Table 1. Amino acid composition of the tryptic activation-site peptide from $N(EtOH)_3$-treated proHisDCase after $NaBH_4$ reduction

Amino acid	Molar ratio	Number of residues*
Thr	2.52	(3)
Ser	0.89	(1)
Glx	0.93	(1)
Gly	1.93	(2)
Ala	1.80	(2)
Val	0.90	(1)
Phe	1.0	(1)
Arg	0.94	(1)

The peptide (10 nmol) was reduced in 50 μl of 1% ammonium bicarbonate (adjusted to pH 8.5 with ammonium hydroxide) by the addition of sodium borohydride (5 μl of a 16 mg/ml solution in 0.01 M NaOH). After 10 min at 4°C, a second addition of sodium borohydride (5 μl) was made. After a further 10 min of incubation, acetone (20 μl) was added to stop the reaction. The sample was lyophilized, redissolved in 1% ammonium bicarbonate, and purified by HPLC as described in Fig. 2B. Three-quarters of the purified sample was sequenced, and the other quarter was subjected to acid hydrolysis in 6 M HCl at 110°C for 18 hr, followed by amino acid analysis on a Glenco automatic amino acid analyzer.
*To the nearest integer.

Histidine-Catalyzed Activation. In contrast to K^+, NH_4^+, and the other amines tested, activation of proHisDCase by L-histidine (Fig. 4A) displays saturation kinetics. A Lineweaver–Burk plot of these data showed a half-maximal rate of activation at 0.65 mM histidine. At low, nonsaturating concentrations of L-histidine (<2.0 mM), activation of the proenzyme was half-order with respect to histidine concentration. Histidine was a particularly effective activator of the proenzyme: 2.5 mM histidine gave approximately the same rate of activation as 25 mM K^+. We attribute the activating effect of L-histidine to its charged α-amino group, since urocanic acid does not catalyze activation.

The Binding of L-Histidine by proHisDCase. The dissociation constant of the proHisDCase-L-histidine complex, calculated from a Scatchard analysis (Fig. 4B) of the equilibrium binding data obtained by the membrane-filter technique described by Lever (22), is 1.7×10^{-4} M; the same data indicated the presence of six binding sites for L-histidine per proenzyme hexamer. The affinity of the proenzyme for L-histidine explains the high rate of activation achieved at low

Table 2. Comparative effects of various amines on the catalysis of activation or inactivation of mutant 3 proHisDCase

Amine cation	Initial rate, %/hr		Dehydroalanine in product I,* residues per subunit
	Activation	Inactivation	
NH_4^+	3.0	0.0	—
$NH_3C(CH_2OH)_3^+$	3.0	0.75	0.88
$NH_3CH_2CH_2OH^+$	3.2	5.0	0.80
$NH_2(CH_2CH_3)_2^+$	1.8	28.0	0.85
$NH(CH_2CH_2OH)_3^+$	2.1	31.0	0.80
$N(CH_3)_4^+$	3.0	10.0	0.82
L-Histidine	2.4	4.2	0.86

Rates of activation and of inactivation were measured in 0.1 M amine at pH 8.0 as shown for $N(CH_2CH_2OH)_3$ in Fig. 1B.
*Dehydroalanine was assayed with 2,4-dinitrophenylhydrazine after conversion to pyruvic acid by mild acid hydrolysis in 3 M HCl at 100°C for 1 hr as described by Patchornik and Sokolovsky (20).

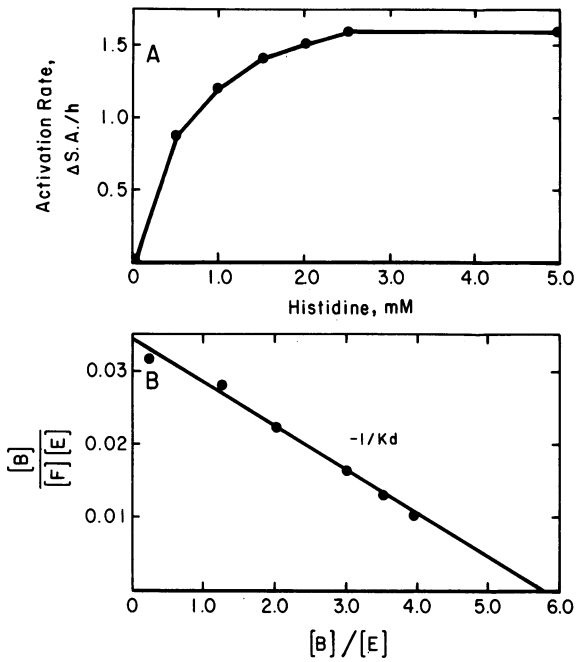

FIG. 4. (*A*) The effect of L-histidine (free base) concentration on the rate of proHisDCase activation at pH 7.5 and 37°C, showing the change of specific activity (S.A.) per hour (h). (*B*) Scatchard analysis of the binding of L-histidine by proHisDCase. Binding data were obtained as described in the text. [B] and [F] represent molar concentrations of bound and free histidine, respectively; [E] is the molar concentration of proHisDCase (π_6), and K_d is the dissociation constant of the histidine–proenzyme complex. The intercept on the horizontal axis indicates the number (six) of L-histidine binding sites per molecule of proenzyme (π_6).

concentrations of histidine and indicates that the pyruvoyl group of HDC, although essential for histidine decarboxylation, does not play an important role in substrate binding. The same conclusion is indicated by the similarity in concentrations of histidine required for a half-maximal rate of proHisDCase activation (0.65 mM) and half-maximal rate of histidine decarboxylation by HisDCase [0.4 mM (8)].

Competitive Inhibition of Histidine-Catalyzed Activation by Monovalent Cations. The activation of proHisDCase by histidine and the other amines was inhibited by monovalent cations such as K^+, NH_4^+, and Na^+ (Fig. 5). Magnesium, a divalent cation, which does not catalyze proenzyme activation, did not inhibit activation of proHisDCase by amines.

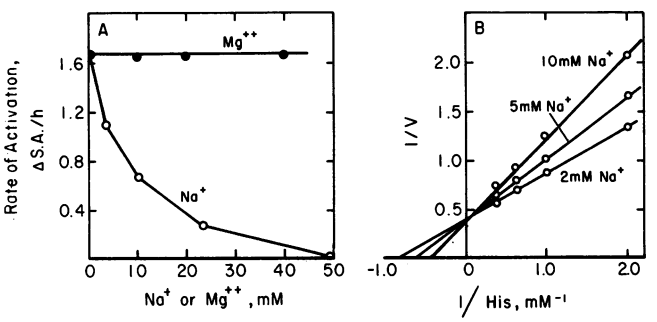

FIG. 5. (*A*) Comparative effects of Na^+ (o—o) and Mg^{2+} (●—●) concentrations on the rate of activation of proHisDCase by L-histidine (5 mM) at pH 7.5. ΔS.A./h represents the change in specific activity per hr. The rates of histidine-catalyzed activation were determined by comparison of the rates of activation in the absence and presence of histidine at each cation concentration. Na^+ and Mg^{2+} were added as their phosphate and chloride salts, respectively. (*B*) Competitive inhibition of histidine-catalyzed activation of proHisDCase by Na^+ at pH 7.5. Rates of histidine-catalyzed activation (v) were determined as described in *A*. See the text for further details.

The inhibitory effect of K^+, NH_4^+, and Na^+ on histidine-catalyzed activation was investigated by measuring the rate of proenzyme activation (v) at a series of histidine concentrations (0.5–2 mM) in the presence of different monovalent cation concentrations (0–15 mM). Plots of $1/v$ versus 1/[histidine], shown for Na^+ in Fig. 5*B*, yielded a family of lines intersecting at a common point on the y-axis in each case. This pattern, characteristic of competitive inhibition, indicates that histidine and monovalent cations exert their activating effect on proHisDCase at the same site. By plotting the apparent "K_m" values obtained for L-histidine vs. the monovalent cation concentrations, apparent K_i values of 2.8 mM (K^+), 4.4 mM (NH_4^+), and 3.5 mM (Na^+) were obtained.

DISCUSSION

Previous studies (5, 23) have shown that conversion of pyruvate-free proHisDCase (π_6) to the pyruvoyl enzyme HisDCase $(\alpha\beta)_6$ occurs by the following overall reaction: $\pi_6 + 6H_2O \rightarrow (\alpha\beta)_6 + 6NH_3$. In the presence of appropriate monovalent cations (K^+ or NH_4^+ are most effective), this reaction is autocatalytic and proceeds essentially to completion at pH 7.6–8.0. The reaction is unusual in that cleavage of the π chain occurs by an intrachain serinolysis, rather than by hydrolysis, with transfer of the side-chain oxygen atom of Ser-82 to the COOH terminus (Ser-81) of the nascent β chain coincident with cleavage of the π chain and conversion of Ser-82 to a pyruvate residue. Two possible mechanisms (routes 1 and 2 of Fig. 6) by which this conversion might occur have been suggested (1). Of these, route 1 was preferred because (*i*) β-elimination of $RCOO^-$ is expected on chemical grounds to be more facile than that of HO^-, and (*ii*) since ^{18}O is transferred quantitatively from Ser-82 to Ser-81 during activation, route 2 requires complete shielding of HO^- from

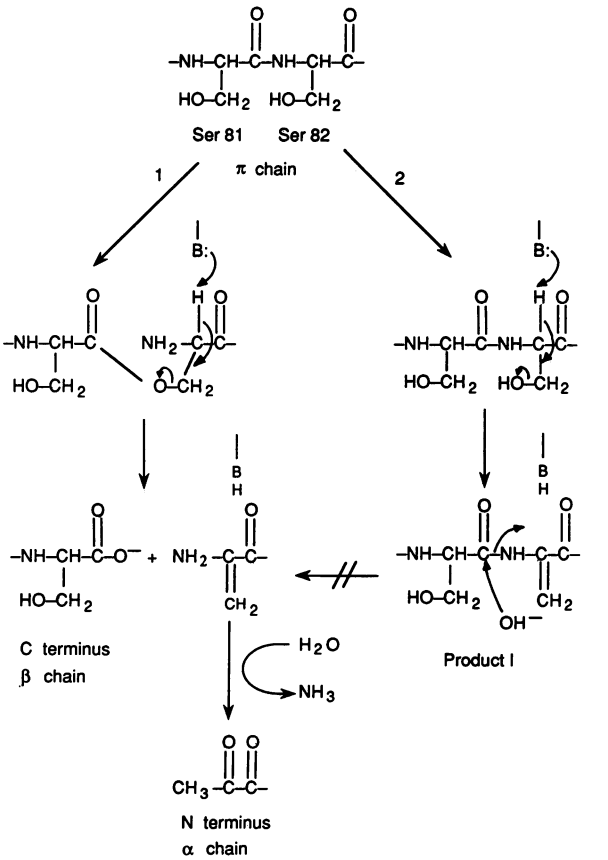

FIG. 6. Postulated route(s) for the activation of proHisDCase (compare ref. 5) and of product I formation. See the text for details.

H_2O, and this appears unlikely in a reaction that requires H_2O at essentially the same locus for the next step in the overall reaction to proceed.

In the studies described here, we found that amines—primary, secondary, tertiary, or quaternary—could substitute for monovalent metal ions in the activation of proHisDCase. However, amine-catalyzed activation differs from the K^+- or NH_4^+-catalyzed process in that complete activation of the proenzyme is not achieved; amines simultaneously catalyze, in a competing reaction, the inactivation of proHisDCase. This inactivation, as demonstrated by sequence analysis of a peptide from $N(EtOH)_3$-inactivated proHisDCase (product I) corresponding to the cleavage site of proHisDCase, results from α,β-elimination at Ser-82 to yield a dehydroalanine residue at this position, thus converting the π chains of proHisDCase to π' chains of product I with identical electrophoretic properties. Only one dehydroalanine residue is present per π' subunit regardless of the amine examined (Table 2), and since the reaction occurs under mild conditions (0.1 M amine at 37°C and pH 6.5 and above) and there was no evidence of denaturation, we assume that Ser-82 is the residue changed in each case.

Since product I is identical with the key intermediate of route 2 in Fig. 6 and does not activate during prolonged incubation with K^+ under optimal activation conditions, route 2 (or any modified route that requires product I as a discrete intermediate) seems to be eliminated as a possible activation mechanism for proHisDCase.

Like HisDCase itself, proHisDCase binds histidine (K_d = 1.7×10^{-4} M; Fig. 4*B*). Therefore, activation of proHisDCase by histidine, unlike that by other amines or by monovalent metal ions, shows saturation kinetics. However, activation by histidine is unlikely to be of physiological significance because it is completely prevented in a competitive manner by low concentrations of K^+, Na^+, or NH_4^+. This inhibition provides a clue as to the nature and location of one site at which monovalent cations are required for the activation of proHisDCase. A comparison of the crystal structures of proHisDCase and HisDCase has revealed that, apart from the absence of the bond between Ser-81 and Ser-82 and a 1.9-Å shift at this position in HisDCase, there is no discernible difference in conformation between the proenzyme and the active enzyme (24). Therefore, the binding sites for histidine in the proenzyme and in the activated proenzyme are likely to be very similar. Since HisDCase binds histidine in such a way that its α-amino group lies in close proximity to the pyruvoyl group, with which it forms a Schiff base during catalysis (8), this α-amino group in the proHisDCase–histidine complex must lie close to Ser-82, the residue from which the pyruvoyl residue is derived. The observation that K^+, NH_4^+, and Na^+ compete with histidine in the activation reaction indicates that its α-amino group and the monovalent cations exert their effects at the same site: in the activation site of the proenzyme, which is in close proximity to the bond between Ser-81 and Ser-82 that is broken during activation. The relatively low K_i values of the monovalent cations ($2.8–4.4 \times 10^{-3}$ M) indicate that this binding site is saturable at low ion concentrations. However, activation of the proenzyme by K^+ does not reach maximum rates even at 2.4 M K^+.

These results are consistent with the presence of two distinct binding sites for monovalent metal ions on the proenzyme: one saturable, near Ser-82, and the other unsaturable. Occupation of the saturable site by alkali metal ions prevents binding of the amino group of histidine or other amines and thus prevents their catalysis of both activation and inactivation of proHisDCase; occupation of both sites is required for maximum rates of activation by metal ions alone to occur. The unsaturable site is probably located near Ser-82 as well, since it appears to be accessible to histidine. A possible role of monovalent cations at one of the sites may be to stabilize the carbonyl anion of Ser-81 (a possible resonance form of this carbonyl group), thereby promoting nucleophilic attack by the hydroxyl oxygen of Ser-82 in reactions leading to ester formation in route 1 of Fig. 6. Binding of monovalent cations to the other site may be necessary to promote α,β-elimination at Ser-82; there is ample precedent for such a requirement by enzymes that catalyze β-elimination reactions (25).

Both route 1 (Fig. 6) leading to activation of proHisDCase, and route 2 leading to product I formation, require an α,β-elimination reaction at Ser-82. It is easy to visualize how minor local distortions of conformation at the activation site, produced by binding of monovalent cations of various sizes, could change the partitioning between the two reactions, as observed (Table 2).

Finally, the observation of reversible binding of monovalent metal cations by proHisDCase at relatively low, physiological concentrations insufficient by themselves to promote rapid conversion of proHisDCase to HisDCase, suggests that the proenzyme itself may play a role other than (or in addition to) its role as a precursor of HisDCase. Such possibilities remain to be studied.

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