Site-Directed Mutagenesis of Intersubunit Boundary Residues in Histidine Decarboxylase, a pH-Dependent Allosteric Enzyme†

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Received January 9, 1995; Revised Manuscript Received February 15, 1995®

ABSTRACT: Histidine decarboxylase (HDC) from *Lactobacillus* 30a forms a trimer around a central cavity or well. Three active sites are formed around the well at the interface of each of two adjacent molecules. HDC exhibits cooperative kinetics at pH 7.6 and can be described in terms of a two-state, T and R, model. At pH 4.8, protons stabilize HDC in the R form. Asp 198 and Asp 53, from a neighboring molecule, are the core of the pH-sensitive mechanism controlling the shift in quaternary state. Eight site-directed mutations have been made to analyze the region. Several mutants, including the conversion of Asp 53 to Asn, cause HDC to exhibit sigmoidal kinetics even at pH 4.8. Others lock the enzyme into the T state. Kinetic analysis suggests that k_{cat} values for T and R states are similar. The K_m for the T state, near 8 mM, exceeds that for the R state by 40-fold and shows HDC is primarily regulated by altering its affinity for substrate.

The *Lactobacilli* are lactic acid producing bacteria that lower the pH of their surroundings as they metabolize. *L.* 30a grows well at pH values between 5 and 6.5, but its metabolism will lower the environmental pH to about 4, where growth stops. In the presence of histidine, wild-type *L.* 30a strains, through the action of histidine decarboxylase (HDC), produce histamine which resists the lowering of pH and allows denser growth than for mutant strains lacking HDC activity (Recsei & Snell, 1972). Studies carried out on *Lactobacillus buchneri* suggest that HDC works in concert with a histidine/histamine antiporter. The net effect of the HDC reaction is to remove a proton from the cytoplasm, thereby raising the pH, and to transport the positive charge outside, as histamine, to create an energy-rich electrostatic membrane potential (Molenaar et al., 1993).

HDC from *Lactobacillus* 30a is synthesized as a 310 residue (34 kDa) proenzyme (π chain). The protein assembles into a trimer, which in turn dimerizes to form a hexamer (Hackert et al., 1981). Activation by a serinolysis reaction cleaves the peptide chain between serines 81 and 82, creating an amino-terminal 81 residue β chain and a 228 residue α chain. A pyruvoyl moiety is formed from Ser 82 at the amino terminus of the α chain, and it acts as the enzyme cofactor for conversion of histidine to histamine and CO_2. The properties of HDC have been reviewed recently (van Poelje & Snell, 1990).

Catalysis by HDC involves formation of a Schiff base between the substrate amine group and the pyruvoyl cofactor. Electrons from the substrate carboxylate flow to the pyruvate sink, allowing the release of CO_2. Histamine is released when water hydrolyzes the Schiff intermediate (Rescei & Snell, 1970). The enzyme shows hyperbolic kinetics at pH values from 3.2 to 6.5. It has roughly constant activity over that pH range; at pH 4.8, HDC has a $K_m \approx 0.3$ mM for His and a $k_{cat} = 2800$ min^{-1} [based on a V_m of 82 μmol min^{-1} (mg of enzyme)$^{-1}$]. Most of the kinetic characterization of HDC has been done at pH 4.8, and this value will be used in the present work. HDC becomes increasingly cooperative as the pH becomes more alkaline. At pH 7.6, it has a Hill number of 2.0, an $S_{0.5}$ of 90 mM, and a $k_{cat} = 1800$ (Rescei & Snell, 1970; Pishko & Robertus, 1993).

The simplest models of cooperative kinetics assume an oligomeric protein possesses at least two tertiary and quaternary forms, a tense (T) and a relaxed (R) state. Each of these quaternary states has a characteristic hyperbolic velocity profile, with a characteristic K_m. A sigmoidal curve arises when substrate, or other effector, causes an enzyme to shift from T state kinetics to the faster R state. The sigmoidal kinetics of HDC are consistent with this notion (McElroy & Robertus, 1989). The K_m values for these two states at pH 7.6 are 50 and 5 mM, respectively (Pishko & Robertus 1993). The T to R transition can be triggered by the substrate histidine acting as a homotropic effector. Low pH also acts as an effector, apparently stabilizing the R state.

The X-ray structure of activated histidine decarboxylase has been solved to high resolution (Parks et al., 1985; Gallagher et al., 1993). Three HDC molecules trimerize, forming a central well with three active sites near the bottom. Two trimers can also form weak tail-to-tail interactions to form a hexamer (Hackert et al., 1981), but it is unlikely that this has any catalytic significance. This is supported by the observation that homologs of the *L.* 30a HDC, like that from *Micrococcus SP.*, exist as trimers (Rashkovetskii & Prozorovskii, 1983). They exhibit the same basic kinetic patterns as the *L.* 30a enzyme, including pH-dependent cooperativity. In this paper, we will assume cooperativity is expressed within the trimer of HDC.

A crucial observation from the X-ray work is that each HDC active site is formed at the interface between two monomers of the trimer. The X-ray structure identified several amino acid side chains in the active site which could be important contributors to the catalytic mechanism. Some

† This work was supported by grants GM35989 and GM30048 from the National Institutes of Health and by grants from the Foundation for Research.

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® Abstract published in *Advance ACS Abstracts,* May 1, 1995.

0006-2960/95/0434-6069$09.00/0

of these groups, like Glu 197, are on the same HDC molecule as that donating the pyruvate, and some residues are donated to the active site by a 3-fold related molecule; the latter residues are given a primed number. The structure of HDC complexed with histidine methyl ester, an inhibitor and substrate analog, revealed that the substrate imidazolium forms an ion pair, or hydrogen bonds, with Asp 63′ and also bonds with the carboxy-terminal carboxylate of the α chain (Ser 81). During binding, the substrate imidazolium was partially covered by Ile 59′, which moved 1.5 Å from its position in the unliganded HDC. The largest motion induced by substrate binding is the 30° rotation of Tyr 62′, moving the hydroxyl group 3.3 Å where it hydrogen bonds with the Ser 81 carboxyl group (Gallagher et al., 1989).

On the basis of the X-ray model, a number of site-directed mutants of HDC have been produced to explore the mode of autoactivation (Vanderslice et al., 1988) and the catalytic mechanism (McElroy & Robertus, 1989; Gelfman et al., 1991; Pishko & Robertus, 1993). For example, conversion of Glu 197 to Gln (E197Q) decreased k_{cat} 8000-fold showing its importance as an acid in the catalytic mechanism (McElroy & Robertus, 1989). Conversion of Asp 63′ to the neutral Asn (D63N) had no effect on k_{cat}, but increased K_m over 250-fold. Because the mutant Asn residue could in principle hydrogen bond to the substrate, these results suggested that in the native enzyme a charge-dependent ion pair was formed (Pishko & Robertus, 1993).

Conversion of Glu 66′ to Gln (E66Q) was very interesting because it created an HDC protein which exhibited sigmoidal kinetics, with a Hill number of 1.5, at both pH 4.8 and pH 7.6 (McElroy & Robertus, 1989). Glu 66′ is donated to the active site of an HDC monomer by a 3-fold related molecule. The essence of cooperativity is that molecules within an aggregate must communicate. The E66Q mutation suggested that Glu 66′ was part of the intermolecular communication network and that the Gln-containing mutant, unlike wild-type, could not be driven completely to the R state, even at pH 4.8.

In this paper, we use site-directed mutagenesis to examine the role of a number of carboxylate residues on the HDC surface. The most important results reveal that Asp 53′ and Asp 198 may be involved in mediating the pH-dependent switch in quaternary structure governing HDC activity.

MATERIALS AND METHODS

Site-directed mutagenesis was carried out as described in Gelfman et al. (1991). Briefly, uracil-containing template was produced by the method of Kunkel (1985). Mutagenic primers 29−33 bases in length and containing the codons for the desired alteration were extended using a variation of the method of Craik et al. (1985). The uracil-containing dsDNA was transformed into a *dut*$^+$ *ung*$^+$ strain, *Escherichia coli* DH5αF′, where RF replication greatly favors the mutant strand over the uracil-containing wild-type strands. Recombinant clones were screened for mutations by DNA sequencing using the appropriate primer from a panel of primers. Potential mutant clones were then sequenced over the length of the gene to be sure only the desired changes had been made. The mutant gene was transferred to a pUC-based plasmid for expression. Protein was isolated as described previously (Copeland et al., 1987; Gelfman et al., 1991).

Mutant enzymes were chemically characterized as described by Gelfman et al. (1991). Briefly, proenzyme autoactivation was followed using gel electrophoresis and densitometry to measure the half-time (τ) of the first-order π chain cleavage (Copeland et al., 1987). Initial rate kinetic data were determined at 37 °C, in a 0.1 M citrate/0.2 M phosphate buffer at pH 4.8 as previously described (Copeland et al., 1987). Kinetics were carried out at pH 7.6 as described by Pishko and Robertus (1993). For hyperbolic kinetics, V_m and K_m values were determined from double-reciprocal plots and also by least-squares nonlinear regression analysis using the program SigmaPlot 5.0 (Jandel Scientific); the methods gave comparable results. V_m was divided by the concentration of cofactor to calculate k_{cat}. For sigmoidal kinetics, $S_{0.5}$ and V_m were determined from nonlinear analysis of the initial rate data and from Hill plots, using SigmaPlot.

Table 1: Autoactivation and Kinetic Parameters for Various HDCs at pH 4.8[a]

protein	τ (h)	k_{cat}/min	K_m or $S_{0.5}$ (mM)	Hill no.	K_m for T state (mM)
wild-type	5	3100 ± 100	0.2 ± 0.04		
D52−54N	6	1200 ± 65	9.7 ± 1.2		
D52,54N	2	3000 ± 230	0.2 ± 0.1		
D53,54N	7	2900 ± 190	9.8 ± 1.5		
D52,53N	10	1300 ± 50	7.7 ± 0.6		
D53N	7	820 ± 15	*2.8 ± 0.2	1.6 ± 0.1	8.5 ± 0.8
D198N	5	1200 ± 25	1.4 ± 0.1		
D53,198N	16	3100 ± 130	*2.6 ± 0.1	1.7 ± 0.1	7.2 ± 1.2
E207−9Q	2	2800 ± 150	0.3 ± 0.1		

[a] τ is the half-time for the first-order autoactivation in 0.8 M K^+, pH 7.6 at 37 °C. The affinity for substrate is measured as K_m for hyperbolic kinetics and $S_{0.5}$ for sigmoidal kinetics; the latter values are marked in the table with an asterisk. The Hill number, a measure of HDC cooperativity for the HDC trimer, and the K_m value for the T state are listed for these cases. The turnover number, k_{cat}, is calculated as V_m/[HDC]. The standard error from the nonlinear parameter fit to kinetic equations is reported in the table.

RESULTS

As part of this project, we have remeasured the initial kinetic rates for wild-type HDC. Recent measurements suggest that V_m values, and consequently k_{cat} values, are very similar to those reported by Recsei and Snell (1970), and Copeland et al. (1987). We find $V_m = 92$ μmol min^{-1} mg^{-1} and $k_{cat} = 3100$/min compared with values of 82 μmol min^{-1} mg^{-1} and 2800/min previously reported. However, K_m values appear to be 0.2 mM, about half the 0.4 mM reported previously.

Three consecutive aspartates, 52′, 53′, and 54′, are found along the surface of the active site well of the HDC trimer. In order to begin studies on the possible significance of these residues, a single mutagenic primer was used to convert all three to asparagines (D52−54N). As seen in Table 1, the neutralization of these three charges has no effect on the autoactivation of the proenzyme, and so we may infer that the residues play no role in that process. The initial rate data for the mutant protein are shown in Figure 1. The profile, at pH 4.8, is clearly hyperbolic, with k_{cat} reduced 3-fold from that of wild-type to about 1200/min; K_m is increased about 50-fold to about 10 mM compared to wild-type (Table 1).

To further analyze the 52′−54′ cluster, the gene for the D52−54N protein was mutated with three separate primers. These converted each Asn residue, in turn, back to the wild-type Asp. The protein in which position 52′ is Asp while

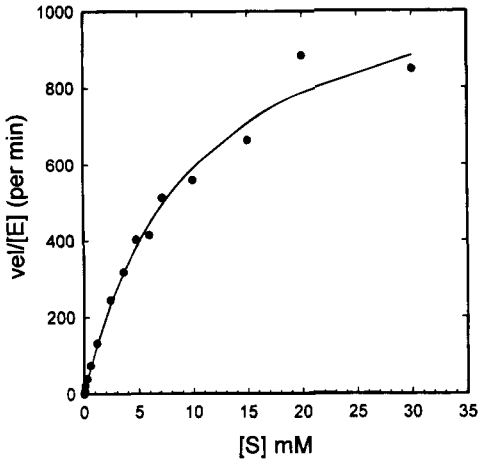

FIGURE 1: Initial rate kinetic data for the D52−54N mutant of HDC. Velocity data were measured at pH 4.8 over the substrate levels indicated.

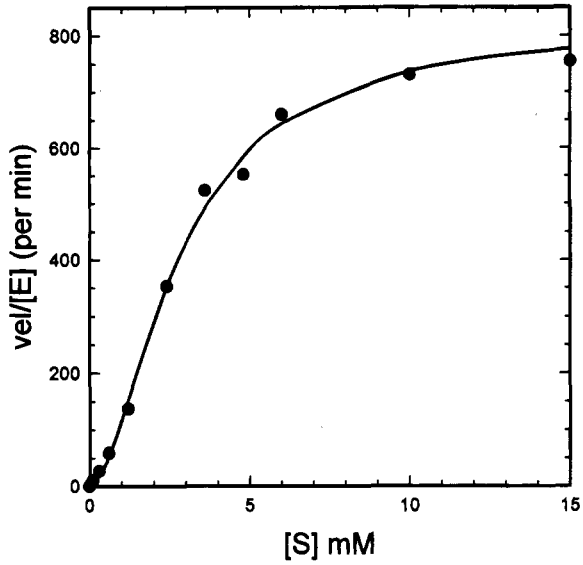

FIGURE 2: Initial rate kinetic data for the D53N mutant of HDC. The velocities were measured at pH 4.8 and exhibit a sigmoidal (cooperative) profile.

retaining Asn at positions 53′ and 54′ is called D53,54N; a similar notation is used for the other double mutants. D52,-54N has hyperbolic K_m and k_{cat} values which are essentially identical to wild-type. That is, neutralizing Asp 52′ and 54′ has no catalytic effect as long as position 53′ is left as aspartate. The other two double mutants involve neutralization of Asp 53′, and each shows significant kinetic changes. D53,54N has a hyperbolic k_{cat} similar to wild-type, but K_m is increased 50-fold. The D52,53N protein shows hyperbolic kinetics in which k_{cat} decreases, roughly 2−3-fold compared with wild-type, to 1300/min while K_m increases 40-fold to about 8 mM. As shown in Table 1, these values are very similar to those of the triple mutant, whose kinetics are illustrated in Figure 1.

Because of the apparent importance of Asp 53′, a single mutation was created converting the residue to Asn (D53N). The kinetic profile is sigmoidal at pH 4.8 (Figure 2). The mutant protein has a k_{cat} = 820/min, $S_{0.5}$ = 2.8 mM, and the Hill number is 1.7.

After the mutagenesis studies described above were well advanced, the 2.5 Å refinement of HDC was completed (Gallagher et al., 1993). It revealed that Asp 53′ was in close

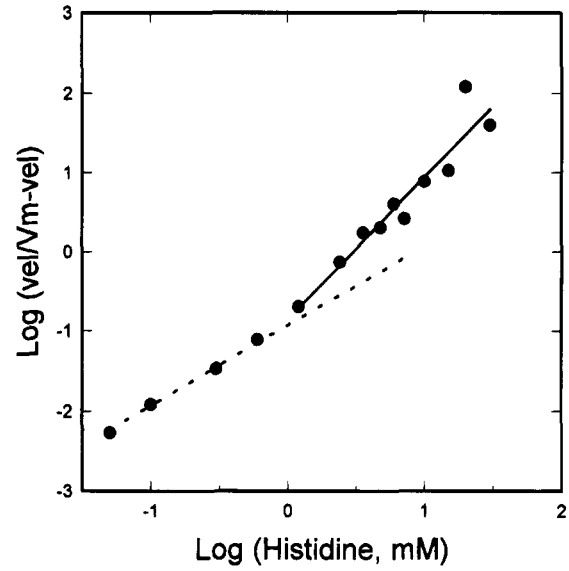

FIGURE 3: Hill plot for D53N HDC at pH 4.8. Velocities at low substrate concentrations have a Hill number (slope) of 1.0. This represents kinetics for the T state. The dashed line is extrapolated to show the apparent K_m for this state is 9 mM. The central slope shows a Hill number of 1.6 for the HDC trimer and $S_{0.5}$ = 3 mM.

contact, across the intermolecular boundary, with Asp 198. Under the pH 4.8 conditions of the crystals, the carboxyl groups are apparently protonated and sharing hydrogen bonds. To investigate the role of Asp 198, it was converted to an Asn (D198N). The purified protein showed apparent hyperbolic kinetics at pH 4.8; k_{cat} = 1200/min and K_m is 1.4 mM, 7-fold above wild-type.

To complete the analysis of this structural region of HDC, a double mutant was constructed in which both Asp 53′ and Asp 198 were converted to amides (D53,198N). This protein exhibited exclusively sigmoidal kinetics at pH 4.8. The k_{cat} value over 3100/min was similar to wild-type, $S_{0.5}$ was 2.6 mM, and the Hill number measured 1.7.

As mentioned above, sigmoidal kinetic profiles can be thought of as arising when an enzyme switches from a T state to a more active R state. Each of these base states may be considered to exhibit hyperbolic kinetics and to have a characteristic K_m value. A Hill plot (Hill, 1913) may be used to compute the Hill number and the K_m values for the T and R states, as has been done for wild-type HDC at pH 7.6 (Pishko & Robertus, 1993). A Hill analysis was performed on each of the sigmoidal profiles described above. Figure 3 shows the plot for the D53N mutant. Velocity data from the low substrate concentrations have a slope of 1, indicating it corresponds to a hyperbolic base (T) state. Extrapolation of the data suggests the K_m for that state is 8.5 mM. Substrate levels could not be made high enough to measure the K_m for the R state. Hill plots for the D53,-198N mutant showed a K_m of 7.2 mM; these values are shown in Table 1.

The 207−209 Carboxylate Cluster. The X-ray model of HDC shows that each monomer has two patches of three consecutive carboxylates on the surface. These are glutamates 52, 53, and 54 in one cluster and Glu 207, Asp 208, and Glu 209 in the second. Viewed down the molecular 3-fold, these create a rough hexagon of negatively charged patches surrounding the central well of the trimer. It was hypothesized that these might help set up an electrostatic field which might guide the positively charged histidine substrate into

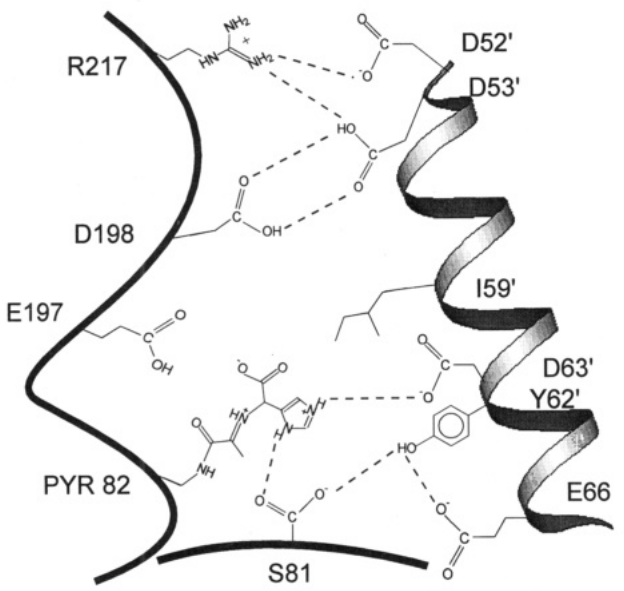

FIGURE 4: Active site and interface area of HDC. This stereo ribbon drawing shows key features of the substrate binding site and the molecular interface thought to be involved in cooperative signaling. The pyruvate cofactor is shown binding a substrate analog, histidine methyl ester (Gallagher et al., 1989). The α-helix is from a symmetry-related molecule. Asp 63′ on the helix ion pairs with the substrate imidazolium. Additional "cross boundary" interactions are seen along the helix interface. In particular, Asp 53′ appears to share protons in hydrogen bonds with Asp 198. These residues are part of a network which also includes Asp 52′ and Arg 217. Mutations of various residues in this interface affect cooperativity.

the central well and toward the active sites at the bottom (Parks, 1984). To help test this contention, a single mutagenic primer was used to convert Glu 207 and Glu 209 to Gln and Asp 208 to Asn (E207−9Q).

The initial rate velocities of the E207−9Q protein show it is catalytically identical to wild type. The mutation has neutralized nine negative charges around the rim of the HDC trimer with no observable effect. This shows that the field hypothesis is not correct and that this cluster of charges has no role in substrate attraction or binding. The charges may play some other role within the cell, e.g., in molecular organization, but that cannot be assayed at the present time. This mutant will not be discussed further.

DISCUSSION

The kinetic measurements made for various mutants allow a fairly straightforward analysis of the two-state model for HDC cooperativity. To facilitate discussion, Figure 4 shows a representation of the molecular structure in the areas likely to affect cooperative kinetics. The figure shows aspects of substrate binding in the HDC active site and structural interactions across the molecular interface. It is based on the X-ray crystallography of HDC at pH 4.8 (Gallagher et al., 1993), and presumably represents the high substrate affinity R state. The figure shows the histidine substrate forming a Schiff base with the pyruvoyl moiety at the amino terminus of the activated HDC α chain. Across the molecular interface is an α helix from a 3-fold related HDC unit; residues along this helix are crucial to the cooperative mechanism of HDC. The imidazolium of the bound substrate is ion paired with the carboxy-terminal carboxylate of Ser 81, and with Asp 63′ from across the molecular interface. Glu 66′ is in a hydrogen bond network with Ser 81. Ile 59′ has moved 1.5 Å to interact with the histidine side chain. Roughly three helical turns upstream from Asp 63′ the figure shows Asp 53′ forming a pair of hydrogen bonds across the molecular interface with Asp 198. This is part of a network which also involves Arg 218 and Asp 52′. At pH 4.8, it is reasonable to assume that Asp 53′ and/or Asp 198 are protonated and this allows close interactions of those moieties across the molecular boundary, as seen by X-ray crystallography. Mutant kinetic data can now be interpreted in terms of the HDC structure.

Wild-type HDC, at pH 4.8, is in the high substrate affinity R state which is illustrated in Figure 4. As shown in Table 1, the wild-type R state shows hyperbolic kinetics with K_m = 0.2 mM and k_{cat} = 3100/min. Mutant proteins D52,54N and E207−9Q behave kinetically like the wild-type. They exhibit hyperbolic kinetics, which means they are locked into one state and do not switch from one quaternary form to another. This is presumably the R form, since the K_m values are identical to those of wild-type. These mutants apparently retain the capacity to be driven to the R state solely by low pH.

However, a pH 4.8 T state must exist for other mutant HDC proteins as seen from the following results.

(1) Conversion of Asp 53′ to Asn alone (D53N) or together with neutralization of Asp 198 (D53,198N) causes the protein to exhibit cooperative, sigmoidal kinetics. This means the mutant enzymes, in the absence of substrate, are in the T state and increasing levels of histidine substrate shift the equilibrium toward an R state. Hill analyses of the sigmoidal data (Figure 3) show the T states have a K_m of about 8 mM (Table 1).

(2) Mutant proteins D52−54N, D52,53N, and D53,54N exhibit purely hyperbolic kinetics with K_m values near 9 mM (Table 1). This value is essentially the same as the K_m for the T state of the cooperative proteins. It is therefore reasonable to assume that D52−54N and D53,54N are locked in the T state at pH 4.8 and cannot shift to the R state even in the presence of high concentrations of histidine.

From the above considerations, it is clear that some HDC mutants, even at pH 4.8, can assume a T and R tertiary and quaternary structure with K_m values of about 0.2 and 8 mM, respectively. It is also possible to analyze the k_{cat} parameters for these states.

The k_{cat} values for wild-type and for mutants in which the R state is stabilized by low pH, like D52,54N, are around 3000/min. The k_{cat} values can be measured from the hyperbolic kinetic profile, and values are listed in Table 1. In a similar vein, the k_{cat} values for T state locked proteins, like D53,54N and D52−54N, can be measured directly; they are 2900 and 1100 per minute, respectively. This suggests that the k_{cat} for T state HDC is quite similar to that for R state HDC; even the 3-fold decrease seen for D53−54N is relatively small. This notion is supported by parameters for those mutants which exhibit sigmoidal kinetics at pH 4.8.

The k_{cat} for the R state of cooperative (sigmoidal) HDCs can also be measured directly from their kinetic plots. At high substrate concentrations, the cooperative enzyme is in the R form and exhibits a maximum velocity appropriate to that state. The R state of D53,198N exhibits a k_{cat} nearly identical to the wild-type. This shows the R state attained by a cooperative HDC can strongly resemble the R state for those HDCs in which the R state is stabilized by pH. The k_{cat} values for other cooperative HDCs show only minor

perturbations from wild-type and D53,198N values. The D53N protein is the most strongly affected, and its k_{cat} is reduced only 4-fold.

We have no unambiguous kinetic measures of k_{cat} for the T state of sigmoidal mutant proteins. However, it again seems reasonable to assume that k_{cat} for the T state of the sigmoidal mutants resembles that for mutants locked in the T state, just as the K_m values for the sigmoidal mutants resemble those for those mutants locked in the T state.

In summary, the k_{cat} values for the T and R states of HDC are very similar, probably around 3000/min. Some mutants show minor variations, generally less than a factor of 4. Given that the mutant proteins used in this study undoubtedly have minor structural perturbations, these changes in kinetic rates are insignificant. On the other hand, the K_m values for the T state exceed those of the R state by 40-fold (8 and 0.2 mM, respectively). It seems clear that HDC is primarily regulated through changes in its affinity for substrate.

The X-ray structure and the above kinetics show that Asp 53′ and Asp 198 are the core of a network which mediates pH-dependent activation of HDC. In particular, any mutation converting Asp 53′ to Asn predisposes the mutant toward the T state. It may be locked there, as in D53,54N or D53–54N, or may be shifted to the R form by high substrate concentrations, as for D53N. As shown in Figure 4, the protein binding network may also includes Arg 217 and Asp 52′, although they are probably of lesser importance. For example, the D52,54N mutant has a kinetic profile indistinguishable from wild-type (Table 1), suggesting Asp 52′ does not strongly govern cooperativity. Nevertheless, a summary of amino acid sequence data from various pyruvoyl-dependent HDCs shows that Asp 52′ as well as Asp 53′ are invariant in the four β chains sequenced and Arg 217 is conserved in both known α chains (van Poelje & Snell, 1990). This conservation may suggest some role in the cooperative mechanism.

In describing the pH-dependent allosterism of HDC, it appears that as the pH rises, protons dissociate from Asp 53′ and Asp 198. Charge–charge repulsion probably causes the Asp 52′–Glu 66′ helix to reorient with respect to the intermolecular boundary and to thereby stabilize the T form of HDC. Kinetic measures demonstrate that such a switch in states must occur. In addition to pH, substrate can also affect the state of HDC. Wild-type HDC is fully cooperative at pH 7.6; this means that the unliganded HDC is in the T state and histidine acts as a homotropic effector to shift it to the R state. At pH 7.6, the T state has a K_m of 50 mM and the R state a K_m of 5 mM (Pishko & Robertus, 1993). The K_m values for both the T and R states at pH 7.6 are higher than the K_m values at pH 4.8. This is partly because the ion pair between Asp 63′ and the substrate imidazolium cannot be made at pH 7.6 where the substrate is uncharged.

At present, we do not understand exactly how histidine acts to stabilize the R state of HDC. The X-ray structure shows that substrate binding triggers the motion of side chains from a symmetry-related molecule, particularly the motions of Ile 59′ and Tyr 62′ (Gallagher et al., 1989). Presumably this motion is involved in the shift from the T to the R state. Site-directed mutation of Glu 66′ (E66Q) affects the T to R transition (McElroy & Robertus, 1989). This site is about three helical turns removed from the Asp 53′ system and probably is not involved with the pH-mediated activation of HDC. Instead, it is part of the network mediating the T to R conversion by substrate binding interactions. In essence, the amino-terminal end of the interface helix shown in Figure 4 (Asp 53′) governs the pH activation while the other end interacts with substrate and mediates the action of the homotropic effector.

The *L.* 30a cells grow optimally near neutral pH; under these conditions, HDC is in the low-affinity, low-activity T state. As the cells grow and secrete lactic acid, the pH drops. Protons bind to the Asp 53′/Asp 198 trap and stabilize the R state. This probably occurs quite readily; it has been shown that HDC is driven to the R form, with a Hill number of 1.0 when the pH drops to 6.5 (Pishko & Robertus, 1993). R form HDC produces histamine to buffer against the acidifying environment (Recsei & Snell, 1972). This pH regulation is almost certainly the main physiological one for HDC. However, if histidine substrate is very concentrated, HDC will shift to the active R form, even at pH 7.6, as shown by its cooperative kinetics at that pH. To date, there is no structural information about the T state of HDC. We have, however, recently crystallized wild-type HDC at pH 7.6, and also the D53,54N mutant which can only assume the T form. These two crystal forms have identical space groups and cell parameters, and each is different from the wild-type R form crystals. The crystals are therefore consistent with the notion of two quaternary forms; the forthcoming X-ray structures should add detail to the kinetic picture presented above.

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BI950045Q