Arginine deiminase (EC 3.5.3.6) catalyzes the hydrolysis of L-arginine to citrulline and ammonium ion; the first step of the L-arginine degradation pathway used by specialized microorganisms as an essential pathway for energy production. The deiminase has attracted much attention because it can be used to reduce nitric oxide levels and therefore serve as a cancer chemotherapeutic agent targeting angiogenesis and tumor growth. With the recent discovery of the arginine degradation pathway in microbial pathogens, arginine deiminase has emerged as a novel drug target. It is within this context that investigations of the structure and the mechanism of catalysis of arginine deiminase from Pseudomonas aeruginosa were initiated in our laboratories.

Arginine deiminase shares structural homology with peptidyl-arginine deiminase, amidinotransferase, and N(G),N(G)-dimethyl-arginine deiminase. Mechanistic studies of this important enzyme family have not been reported. Each of these enzymes possesses a “Cys-His-Glu” motif in their active sites. The conserved cysteine (Cys) residue may mediate group transfer from the guanidinium centers of the respective substrates, as is illustrated with arginine deiminase in Scheme 2. The use of an enzyme active-site Cys residue in nucleophilic catalysis of group-transfer reactions is well preceded.

The possibility of Cys nucleophilic catalysis has important implications for the development of arginine deiminase inhibitors, where design strategies may center on the use of mechanism-based, irreversible inhibitors. In this communication, we report the results from transient-state kinetic experiments, which show that arginine deiminase catalysis proceeds via a covalent adduct formed by active-site Cys406 displacement of ammonia from the guanidinium carbon of L-arginine.

The enzyme was obtained for kinetic study by overexpression of the cloned gene in Escherichia coli. Two site-directed mutants, in which the active-site Cys406 was replaced with serine (Ser) (OH replaces SH functionality) or with alanine (Ala) (CH3 replaces CH2 - SH side chain), were prepared by PCR to serve as controls in the kinetic experiments. The initial velocity of citrulline formation and decay of 14C-labeled enzyme (Figure 1B), were fitted to a first-order rate equation to yield apparent rate constants kcat. The level of [14C]-labeled enzyme (isolated by CCl4-induced precipitation) of [14C]-labeled enzyme (isolated by CCl4-induced precipitation) (Figure 1). The reaction was carried out at 25 °C and pH 5.6 with enzyme in ∼50-fold excess to substrate. If the Kd is used as an estimate of the dissociation constant for the enzyme−substrate complex, ∼80% of the [14C]-labeled arginine is predicted to be associated with enzyme upon mixing. The citrulline formation and arginine decay curves, shown in Figure 1A, were fitted to a first-order rate equation to yield apparent rate constants k = 3.6 ± 0.1 s−1 and k = 4.2 ± 0.1 s−1, respectively. The time courses for the formation and decay of 14C-labeled enzyme (Figure 1B), were fitted using the simulation program KINSIM and the kinetic model shown in Figure 1B to define the rate constant of intermediate formation as 13 s−1 and the rate constant for intermediate consumption as 6.5 s−1. At 120 ms, the intermediate reaches a maximal level of 7% of the estimated level of bound substrate. The 14C-labeled enzyme is judged to be a kinetically competent intermediate, because it is formed faster than is product, and it is consumed at a rate consistent with that of product formation. The level of function.
ates13), thus indicating that its rate of formation is not significantly maximum accumulation of the intermediate is small (typical of what Figure 2.

produce14C-labeled enzyme. The results obtained from studies of mutant enzymes with limiting [14C-1]L-arginine under the same produce14C-labeled enzyme. For this reaction of wild-type arginine deiminase (blue) and Cys406Ser arginine deiminase (green). The red curve was obtained by fitting the data points to the kinetic model shown by using the program KINSIM and [P] and [P] are the substrate and product concentrations at time “t.” (B) Time courses for the formation and decay of the 14C-labeled enzyme from the reaction of wild-type arginine deiminase (blue) and Cys406Ser arginine deiminase (green). The maximum accumulation of the intermediate is small (typical of what is observed for the accumulation of covalent enzyme intermediates), thus indicating that its rate of formation is not significantly faster than its rate of reaction. This is also apparent from the substrate decay curve, which largely mirrors the product formation curve.

The next step was to show that the Cys406 residue plays an essential role in the formation of the reaction intermediate, as is prescribed by the proposed chemical pathway (Scheme 2). For this purpose the Cys406Ala and Cys406Ser mutants were employed. As reported earlier, the two mutants do not catalyze product formation. The chemical pathway also predicts that neither mutant will catalyze the formation of 14C-labeled enzyme. Reaction of the mutant enzymes with limiting [14C-1]L-arginine under the same conditions used for the wild-type enzyme (Figure 1B) failed to produce 14C-labeled enzyme. The results obtained from studies of the Cys406Ser mutant (shown in Figure 1B) were matched by those observed for the Cys406Ala (data not shown). The conclusion drawn from these observations is that the Cys406 thiol group is essential for the formation of the reaction intermediate.

The X-ray structure of the arginine deiminase active site (see Figure 2) suggests that replacement of Cys406 with a Ser residue will not perturb the active-site residues. Thus, failure of the Cys406Ser mutant to form the reaction intermediate can be attributed to the removal of the active-site nucleophile. Moreover, the X-ray structure and modeled arginine show that the Cys406 thiol group is perfectly oriented for attack at the guanidinium carbon of the L-arginine as it is held in place by H-bonds formed with several active-site residues. His278 is the probable base.

In summary, the close proximity of the Cys406 thiol to the guanidinium carbon of the L-arginine substrate, coupled with the essential role that it plays in the formation of the reaction intermediate, serves as strong evidence to support the proposed role of Cys406 in nucleophilic catalysis. Nucleophilic catalysis in arginine deiminase has important implications for the mechanisms of catalysis in other members of the same enzyme family, which also conserve the active-site Cys. In addition, the results suggest that arginine deiminase inhibitor design strategies may be based on mechanism-based, covalent modification of the active-site Cys406.

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References