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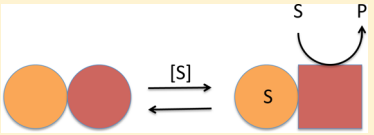
Kinetic Evidence of a Noncatalytic Substrate Binding Site That Regulates Activity in *Legionella pneumophila* L-Serine Dehydratase

Gregory A. Grant*

Department of Developmental Biology and Department of Medicine, Washington University School of Medicine, 660 South Euclid Avenue, Box 8103, St. Louis, Missouri 63110, United States

Supporting Information

ABSTRACT: The L-serine dehydratase from *Legionella pneumophila* (*lp*LSD) has recently been shown to contain a domain (β domain) that has a high degree of structural homology with the ASB domain of D-3-phosphoglycerate dehydrogenase (PGDH) from *Mycobacterium tuberculosis*. Furthermore, this domain has been shown by sequence homology to be present in all bacterial L-serine dehydratases that utilize an Fe−S catalytic center. In PGDH, L-serine binds to the ACT domain to inhibit catalytic activity. However, substrate must be bound to the ASB domain for serine to exert its effect. As such, the ASB domain acts as a codomain for the action of L-serine. Pre-steady-state kinetic analysis of L-serine binding to *lp*LSD demonstrates that L-serine binds to a second noncatalytic site and produces a conformational change in the enzyme. The rate of this conformational change is too slow for its participation in the catalytic cycle but rather occurs prior to catalysis to produce an activated form of the enzyme. That the conformational change must occur prior to catalysis is shown by a lag in the production of product that exhibits essentially the same rate constant as the conformational change. The second, noncatalytic site for L-serine is likely to be the ASB domain (β domain) of *lp*LSD that functions in a manner similar to that in PGDH. A mechanism whose overall effect is to keep L-serine levels from accumulating to high levels while not completely depleting the L-serine pool in the bacterial cell is proposed.



L-Serine dehydratase (EC 4.3.1.17) from *Legionella pneumophila* (*lp*LSD) is a Type 2[1,2] iron−sulfur dehydratase. The incorporation of an iron−sulfur center at its active site sets it apart from mammalian L- and D-serine dehydratases and bacterial D-serine dehydratases that utilize a pyridoxyl 5′-phosphate prosthetic group.[3−6] L-Serine dehydratases convert L-serine to pyruvate and ammonia. This transformation is thought to take place[7−9] by the initial dehydration of serine to yield aminoacrylate, which forms an ene-imine tautomer as depicted in Scheme 1. The imine is subsequently rehydrated to form pyruvate and ammonia.

Rather than these enzymes mainly being involved in the production of pyruvate for gluconeogenesis, recent evidence suggests that these iron−sulfur cluster-containing L-serine dehydratases may function in bacteria in the regulation of L-serine levels that, if too high, may interfere with cell wall synthesis.[10,11] These studies showed that deletion of the genes for L-serine dehydratases in *Escherichia coli* resulted in significant morphological abnormalities and cell lysis. As a result, it was hypothesized that the L-serine dehydratases were necessary to keep L-serine levels sufficiently low so that they would not interfere with incorporation of alanine into the cell wall by the *mur*C-encoded ligase, UDP-*N*-acetylmuramoyl:L-alanine ligase.

*lp*LSD is composed of a single polypeptide chain with two distinct domains, termed α and β. The α domain contains the iron−sulfur catalytic center, while the β domain may function in the regulation of catalytic activity. Evidence of the latter comes from its remarkable structural homology to the allosteric substrate binding (ASB) domain of *Mycobacterium tuberculosis* D-3-phosphoglycerate dehydrogenase (*mt*PGDH).[12] PGDH catalyzes an early step in the biosynthesis of L-serine by utilizing phosphoglycerate from glycolysis.[13] L-Serine is the product of the third enzyme in the pathway, phosphoserine phosphatase, and feeds back to bind to the ACT domain of PGDH as a regulatory effector molecule.[14−17] In *mt*PGDH, the ASB domain contains a second noncatalytic binding site for its substrate whose occupancy is necessary for feedback regulation of activity by L-serine.[12,18] Because of the homology with the ASB domain of PGDH, it has been suggested that the β domain of *lp*LSD may also harbor a second, noncatalytic binding site for its substrate, L-serine.[1] Additional evidence for this idea comes from initial kinetic studies that show that pyruvate can activate enzyme activity and that the activation is competitive with L-serine.[1,2] Because activation is not consistent with pyruvate being a competitive inhibitor for L-serine at the active site, it suggests the presence of a second serine binding site that is capable of modulating enzyme activity upon ligand binding. Because high concentrations of L-serine are capable of eliminating the activation,[2] the natural ligand for the second site may be L-serine. That the same ligand is both binding to a noncatalytic site and turning over at the active site makes it somewhat problematic to study. While the exact location of the second site will eventually be determined by structural analysis and site-specific mutagenesis, this investigation provides pre-steady-state kinetic evidence of the existence of a second substrate binding site within *lp*LSD and develops a plausible

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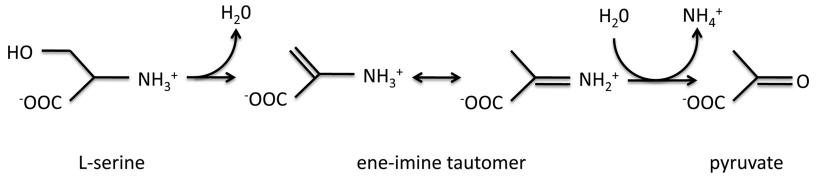
Scheme 1. Proposed Reaction for the Conversion of L-Serine to Pyruvate and Ammonia by Bacterial L-Serine Dehydratases[a]



[a]The dehydration of L-serine results in the production of aminoacrylate, which is in tautomeric equilibrium with the imine form that is proposed to rehydrate to form pyruvate with the elimination of ammonia.

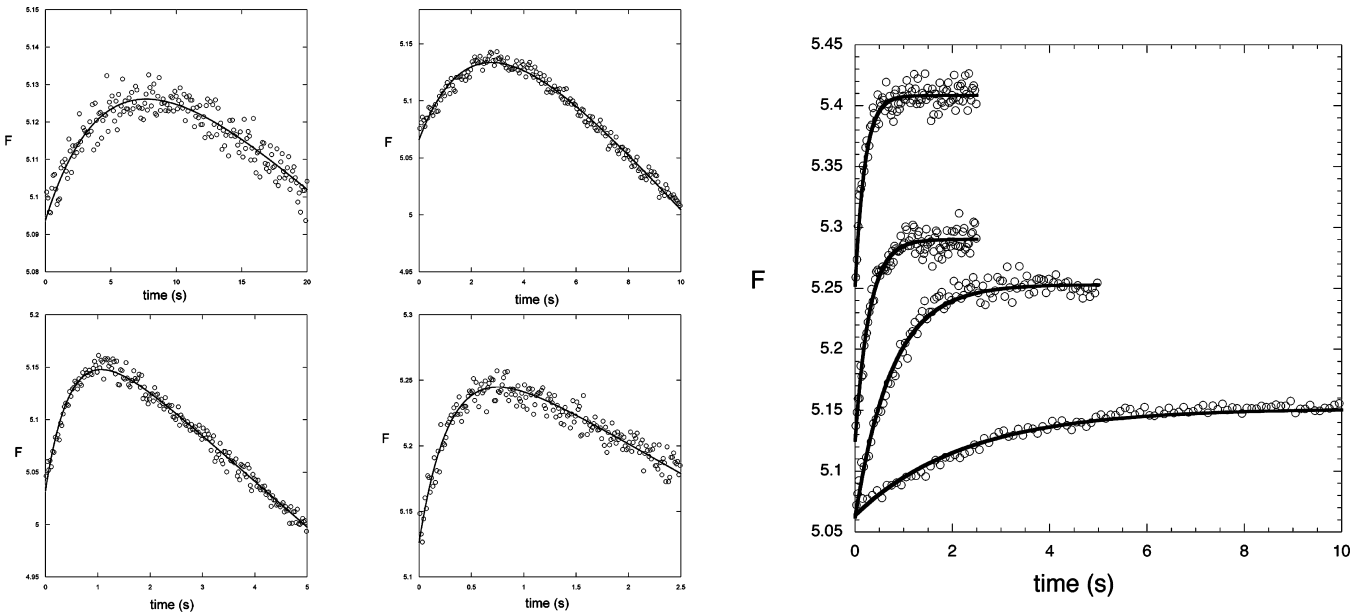


Figure 1. Representative tryptophan fluorescence transients. The enzyme is excited at 295 nm, and the change in tryptophan fluorescence (F) is monitored at >320 nm as a function of L-serine concentration. The left and middle plots are the experimentally observed transients. The L-serine concentrations are 1 mM (top left), 5 mM (top right), 40 mM (bottom left), and 80 mM (bottom right). The data are represented as symbols, and the solid line shows the fit to the appropriate equation. Only every 50th data point is shown for the sake of clarity. The plot on the right shows selected fluorescent transients after subtraction of the linear portion of the response. The L-serine concentrations, from bottom to top, are 2, 20, 80, and 400 mM, respectively. The data are represented as symbols, and the solid line shows the fit to the appropriate equation. Only every 50th data point for 1 mM serine and every 100th data point for the others are shown for the sake of clarity.

mechanism for how it participates in the regulation of enzyme activity.

EXPERIMENTAL PROCEDURES

L-Serine dehydratase from *L. pneumophila* was expressed with a 5′,6-His extension and purified on cobalt-based Talon immobilized metal affinity columns (IMAC) as described previously.[2] Cells were grown in LB medium until the A_{600} reached 0.5−0.7 (2−3 h), and expression was induced with 3 mM IPTG until the A_{600} was between 1 and 3 (4−5 h). The cells were recovered by centrifugation, suspended in 50 mM NaMOPS buffer (pH 7.0), and placed in an anaerobic chamber. The cells were lysed by sonication in the presence of 0.16 mg/mL lysozyme, stirred for 10−20 min, and then treated with 5 mg of DNAase. The supernatant was recovered after centrifugation and loaded onto the IMAC column. Steady-state activity was measured by monitoring the absorbance due to pyruvate production at 250 nm using an extinction coefficient of 93.3 M^{-1} cm^{-1}. The concentration of the active enzyme was determined from the absorbance of the iron−sulfur center at 400 nm using an extinction coefficient of 13750 M^{-1} cm^{-1}.[19] L-Serine was purchased from Sigma-Aldrich.

Anaerobic procedures were performed in a Coy Laboratories type B chamber maintained with an atmosphere of 95% nitrogen and 5% hydrogen. Residual oxygen was removed with a palladium catalyst, and water was removed with an alumina desiccant. Solutions were degassed by being sparged with nitrogen and stirred for 24 h after being introduced into the chamber. Enzyme samples were removed in sealed vials and diluted with oxygen-free buffer solutions just prior to introduction into the stopped-flow syringes. Under these conditions, the enzyme retained activity for several hours.[2]

Gel filtration was performed on *lp*LSD with a calibrated 1.6 cm × 90 cm column of Sephacryl S-200 in 50 mM MOPS buffer (pH 7.0).

Stopped-Flow Fluorescence Spectroscopy. Pre-steady-state kinetic analyses were performed with an Applied Photophysics model SX-20 stopped-flow spectrometer. The reaction mixtures and all reagents were thermostated at 25 °C with a circulating water bath. Serine binding was monitored by following the change in the fluorescence of tryptophan with a 320 nm cutoff filter after excitation at 295 nm. All reactions were performed in 50 mM NaMOPS buffer (pH 7.0). A total of 1000 points were collected for each trace, and 5−10 individual traces were averaged under each set of conditions. The binding

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transients were analyzed with the data fitting function of KinTek Global Kinetic Explorer[20,21] and fit to a single-exponential function plus a linear function defined as

$$Y = A\ \exp(-k_{obs}t) + k_{lin} + C \quad (1)$$

where Y is the fluorescence intensity at time t, k_{obs} is the observed rate of the exponential process, A is the amplitude of the exponential process, k_{lin} is the observed rate of the linear process, and C is an offset value. The k_{lin} term was deleted for fitting of transients where the linear signal was subtracted.

Hyperbolic plots of the observed rates (k_{obs}) versus ligand concentration were fit to

$$k_{obs} = (k_2[L])/(K_d + [L]) + k_{-2} \quad (2)$$

where K_d is the dissociation constant for binding and k_2 and k_{-2} are the forward and reverse rate constants of a subsequent rate-limiting step, respectively.[22,23]

The data in Figure 2 were fit to the equation for a hyperbola with an exponential parameter to account for possible cooperativity

$$A = (A_m[L]^n)/(K_{0.5}{}^n + [L]^n) \quad (3)$$

where A is the amplitude at any ligand concentration [L], A_m is the maximal amplitude, $K_{0.5}$ is the serine concentration at half-A_m, and n is the Hill coefficient. A form of this equation in which A is replaced with velocity (v) and A_m with maximal velocity (V_m) is used for fitting to velocity data.

RESULTS

*lp*LSD contains only a single tryptophan residue that resides in its α domain. When the tryptophan fluorescence is monitored as a function of serine concentration in a stopped-flow spectrophotometer after rapid mixing of enzyme and L-serine, an initial increase in fluorescence is observed followed by a linear decrease in fluorescence (Figure 1). The transients fit best to a single exponential for the initial increase in fluorescence plus a linear function for the subsequent decrease in fluorescence as shown in Figure 1. Because pyruvate absorbs in the 320−360 nm range, the linear portion of the transient is likely due to the steady-state production of pyruvate. The overall signal does not represent a burst response because the extinction coefficient of pyruvate is too low to allow the observation of enzyme-bound pyruvate at the enzyme concentrations used.

Analysis of the observed rate constant for the linear portion of the kinetic transients as a function of serine concentration (Figure 2) yields an apparent K_m for L-serine of 4.3 ± 0.4 mM, which is similar to the value of 5.1 ± 0.1 mM determined from steady-state analysis.[2] Because the steady-state absorbance by pyruvate is linear, it can easily be subtracted so that only the binding transient is observed (Figure 1). When the observed rate constants for the exponential process are plotted as a function of serine concentration, a hyperbolic plot results (Figure 3). This indicates a ligand binding process in which rapid equilibrium binding is followed by a rate-limiting step, usually associated with a conformational change. When the data are fit to eq 2, the fitting statistics for K_d and k_2 are acceptable, but that for k_{-2} is poor (Table 1).

The value for k_{-2} was resolved by globally fitting the data using KinTek Global Kinetic Explorer Pro to a model representing a binding event followed by a conformational transformation (Scheme 2). This simulation yielded a value of 0.3 s^{-1} for k_{-2} that was well constrained between a lower and upper bound as determined by KinTek Global Kinetic Explorer Fit Space analysis (Table 2).

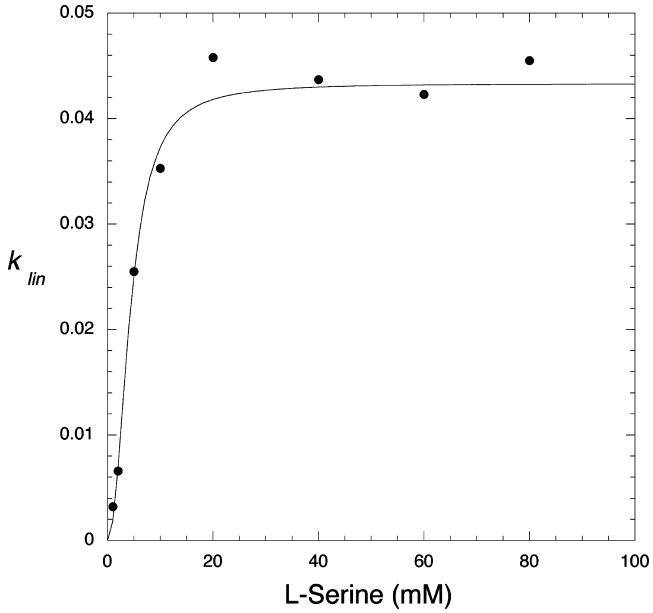

Figure 2. Observed rates for the production of pyruvate. The observed rates from the linear portion of the fluorescent transients are plotted vs serine concentration and fit to eq 3. The data are the symbols, and the fit is represented by the solid line.

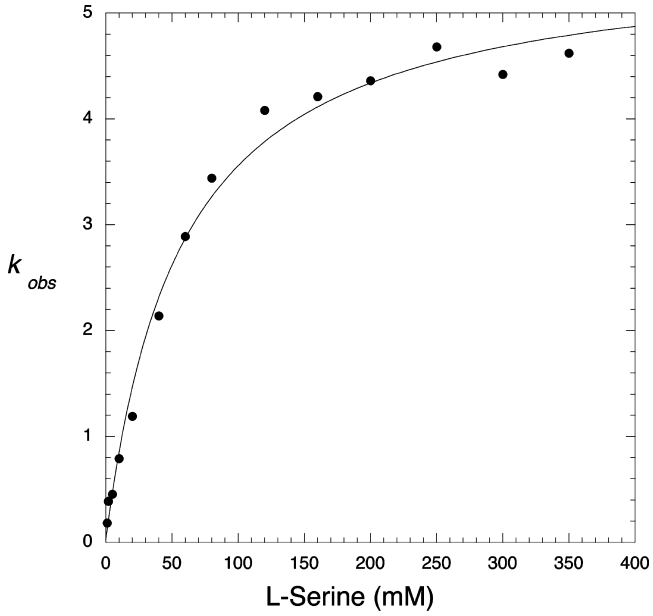

Figure 3. Observed rates for serine binding. The observed rates from the exponential portion of the fluorescent transients are plotted vs serine concentration and fit to eq 2. The data are the symbols, and the fit is represented by the solid line.

Table 1. Kinetic Parameters from Fitting of Observed Rates versus Serine Concentration[a]

K_d	56.8 + 8.3 mM
k_2	5.5 ± 0.2 s^{-1}
k_{-2}	0.03 ± 0.12 s^{-1}

[a]Direct fitting of data from Figure 3 to eq 2.

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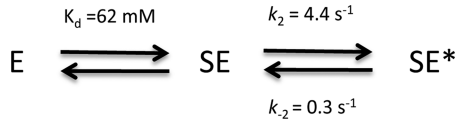
Scheme 2. Depiction of the Binding of L-Serine to a Noncatalytic Site[a]



[a]The noncatalytic site is represented by placing the symbol for the ligand (S) on the left side of the symbol for the enzyme (E), represented as SE. Binding at the catalytic site would be represented as ES (see Scheme 3). E* indicates an activated or conformationally changed form of the enzyme. The rate parameters determined from the kinetic transients are indicated.

Table 2. Kinetic Parameters from Global Fitting of Data

		lower bound	upper bound
K_d	61.8 mM	54.5	75.5
k_2	4.77 ± 0.08 s^{-1}	3.55	6.67
k_{-2}	0.297 ± 0.003 s^{-1}	0.253	0.344

The k_{cat} and K_m for *lp*LSD in 50 mM NaMOPS buffer were previously determined to be approximately 280 s^{-1} and 5 mM, respectively.[2] Because the k_{cat} defines the minimal rate constant for catalysis, the value for k_2 in Table 2 is inconsistent with the process being on the main reaction pathway. This suggested that the process being observed was separate from the catalytic mechanism and most probably represents a second serine binding process at a location separate from the catalytic site. Thus, the fluorescence transients were composed of two separate processes: (1) concentration-dependent binding of L-serine to a second, noncatalytic, site (Scheme 2) and (2) production of product at the catalytic site.

It is also possible that the slow step could represent serine-induced dimer formation. This seemed unlikely because the Type 2 enzyme from *Escherichia coli*[9] was shown to be a dimer in the absence of substrate. To confirm this for the *lp*LSD, another Type 2 enzyme, gel filtration analysis was performed. The results (Figure S1 of the Supporting Information) showed a main peak at 105 kDa and a following shoulder estimated at 50 kDa. These values are in good agreement with the calculated values of 98952 and 49476 Da for the dimer and monomer, respectively. Furthermore, only the peak associated with the dimer displayed catalytic activity. This indicated that, in the absence of substrate, *lp*LSD is predominately a dimer and that the monomeric form does not possess enzymatic activity.

At first, the K_d of 62 mM seemed very high for this to be a physiologically relevant process. However, because the initial binding event is followed by a conformational change and k_2 is significantly larger than k_{-2}, a significant amount of the SE complex would be expected to form and participate in the catalytic cycle until substrate is significantly depleted. Simulation with Kin-Tek Explorer shows that, if the production of SE is considered alone, 50% of the enzyme would be present in the SE complex, at the initiation of the catalytic cycle, at 5 mM substrate (Figure 4). This value is in the same range as the measured K_m for the catalytic reaction.

If a relatively slow noncatalytic binding process were occurring prior to the initiation of catalysis, one would predict to see a lag in the pre-steady-state production of product. When the production of pyruvate is monitored by the absorbance at 250 nm, after rapid mixing of enzyme and substrate in the stopped-flow instrument, a lag is indeed observed prior to the steady-state production of pyruvate (Figure 5). The duration of the lag was found to be dependent on the concentration of L-serine. Fitting of the lag transients to a single exponential with a linear process produced observed rates for the exponential process that, when plotted as a function of serine concentration, produced a nonlinear plot (Figure 6). These data could be fit with eq 2 to yield a K_d of 47 ± 18 mM and a forward rate

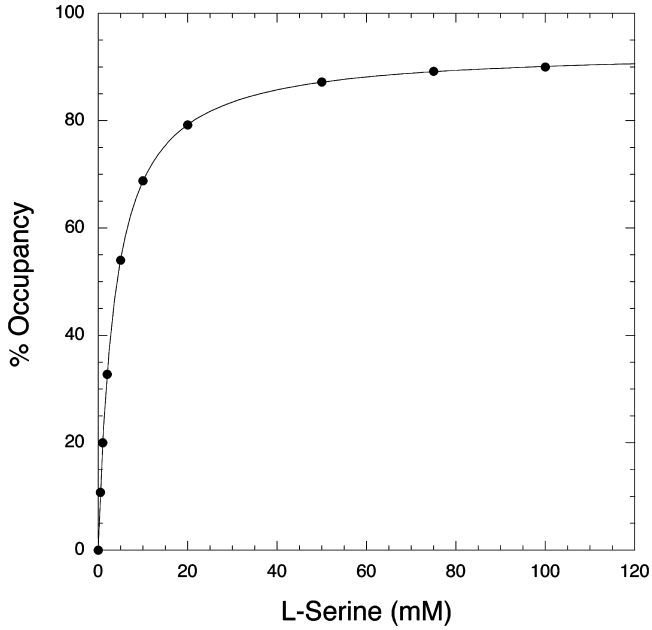


Figure 4. Simulation of the occupancy of E* with L-serine in the absence of turnover. The occupancy of E* with serine at the noncatalytic site was simulated with KinTek Global Explorer based on the parameters determined from Figure 3 and listed in Table 2. The simulation data are depicted with symbols, and the solid line is the fit to the equation for a hyperbola.

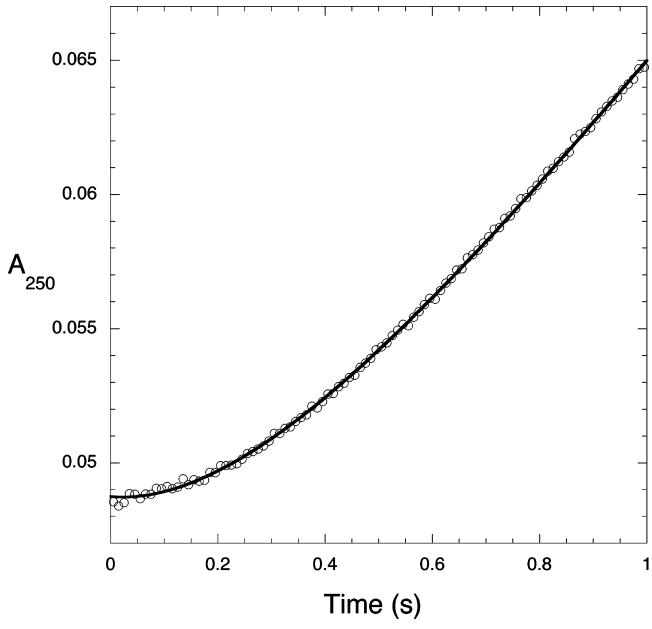


Figure 5. Stopped-flow transient analysis of pyruvate production. The production of pyruvate is monitored at 250 nm after the rapid mixing of enzyme and substrate, L-serine. Every 100th point is plotted. The data shown are for a serine concentration of 50 mM.

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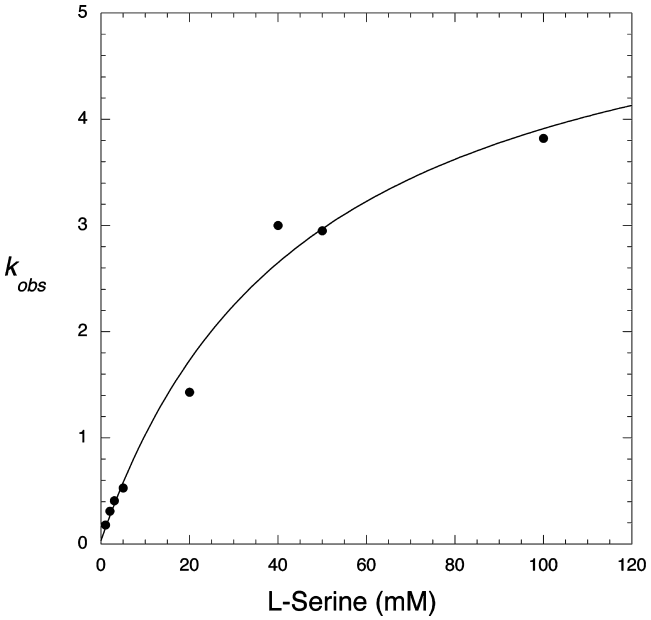


Figure 6. Observed rates of pyruvate production. The observed rates of the lag in pyruvate production are plotted vs L-serine concentration. The data are the symbols, and the line is the fit to eq 2.

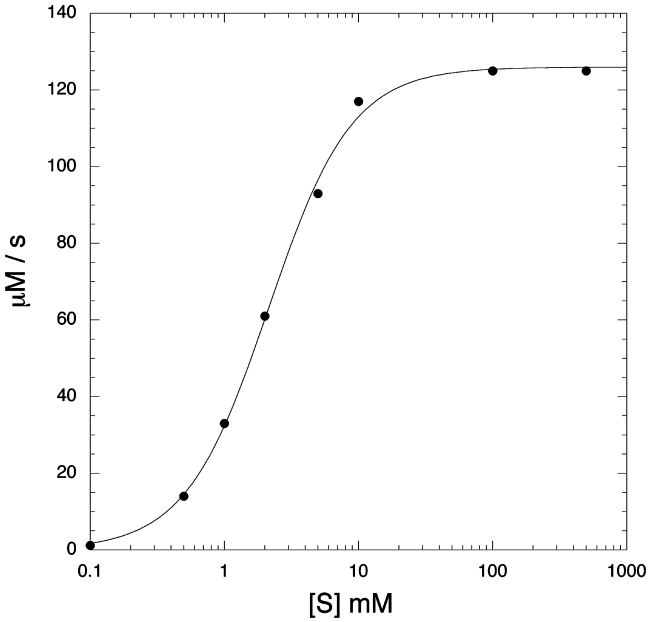
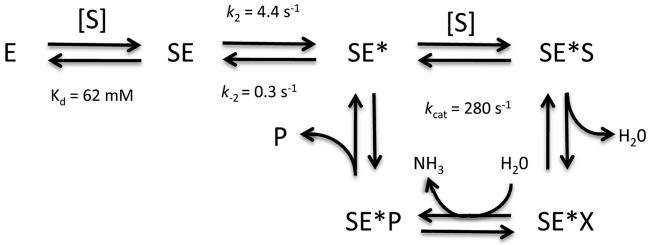


Figure 7. Simulation of Scheme 3. The initial velocities, expressed as micromolar per second, as a function of L-serine concentration are plotted from a simulation of Scheme 3 with KinTek Global Explorer. The initial velocities are represented by the symbols, and the line is a fit to eq 3.

constant of 5.7 ± 0.9 s^{-1}. Both values are similar to those determined for the noncatalytic binding process (62 mM and 4.8 s^{-1}, respectively) shown in Scheme 2 and Table 2.

To gain an appreciation of how the mechanism shown in Scheme 3 would regulate the levels of L-serine, a simulation was performed with KinTek Gobal Explorer. The values determined for the noncatalytic binding process were used, and rates for the catalytic process were set equal to the k_{cat} for the overall process. For the catalytic substrate binding step, a K_d of 5 mM was used. Figure 7 shows the initial velocity of pyruvate production as a function of L-serine concentration according to the model in Scheme 3. The data are fit to eq 3, where A now represents v (velocity) and A_m now represents V_m (maximal velocity). The plot demonstrates an approximate 10-fold difference in the initial velocity comparing concentrations in the 0−400 μM range to those in the 10−20 mM range. The Hill coefficient from the fit is 1.41 ± 0.08, which is comparable to that (1.41 ± 0.04) from independent steady-state kinetic analysis of *lp*LSD.[2]

Scheme 3. Proposed Model for L-Serine Binding to *L. pneumophila* L-Serine Dehydratase[a]



[a]The binding to the noncatalytic site is depicted by placing the symbol for the ligand on the left and that for the catalytic site on the right of the symbol for the enzyme (E). E* represents an enzyme species that has undergone a conformational change as a result of substrate binding to the noncatalytic site. X represents a reaction intermediate, and P represents product.

DISCUSSION

*lp*LSD contains a single tryptophan residue that is located in the α domain of the protein. Rapid mixing with L-serine produces a transient change in the tryptophan fluorescence of the protein. The transients can be separated into two parts, an increase in the fluorescence associated with L-serine binding and a subsequent decrease in fluorescence associated with an absorbance due to the steady-state production of pyruvate. The first portion fits well to a single exponential, while the latter displays a linear response. Analysis of the exponential portion of the transients yields a nonlinear relationship between the observed rates and L-serine concentration, indicating that binding of the ligand is followed by a second, rate-limiting step. Interestingly, the first-order rate constant for the rate-limiting step is significantly smaller than the overall k_{cat} of the steady-state reaction. Because the k_{cat} represents a minimal rate for steps other than that involving ligand binding, the process monitored by the transient kinetic analysis cannot be part of the catalytic cycle. Direct monitoring of pyruvate production in the stopped-flow instrument demonstrates that there is a concentration-dependent lag in the appearance of pyruvate, consistent with the occurrence of a slow step prior to catalytic turnover. The concentration dependence of the lag is hyperbolic and yields a forward rate constant very similar to that determined from the tryptophan fluorescence analysis. These observations support a model in which substrate binds to a site that induces a conformational change prior to the initiation of catalytic turnover. Furthermore, the data support the conclusion that the first site is separate from the catalytic site. If substrate were to bind to the catalytic site and induce a conformational change before turnover occurred, it would be part of the catalytic cycle and would have to have a rate equal to

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or greater than the k_{cat} of the reaction. This model could be considered a type of hysteresis[25] in the sense that a slow transition precedes rapid turnover of the substrate. However, in most hysteretic models in which substrate induces a conformational change, it also turns over. The earlier observations that pyruvate can act as an activator but is competitive with L-serine[1,2] support the existence of a second site for serine binding that is not an active site.

A monomer to dimer transition in response to serine can be ruled out as a possible source of the slow step because the dimer exists in the absence of substrate and only the dimer is active. By analogy to the ASB domain in *M. tuberculosis* phosphoglycerate dehydrogenase,[12,18] it is likely that the serine binds at the interface between the two β domains in the dimer.

The model depicted in Scheme 3 combines the relatively slow process measured by the stopped-flow analysis described above and the relatively fast turnover of substrate as required by the steady-state k_{cat}. The model depicts two serine binding sites, one noncatalytic site for the production of SE* and a catalytic site for the binding of a second molecule of substrate to SE* to produce SE*S, which goes on to produce product. E* represents the formation of a catalytically competent enzyme form. In this way, the slow process does not participate in the reaction cycle but must occur before the reaction proceeds. Once E* is formed by substrate binding to the noncatalytic site, it persists and catalyzes turnover of substrate to product.

Like the case with many of the pyridoxyl phosphate-based dehydratases, it is often assumed that the imine is rehydrated nonenzymatically after it is released from the enzyme. However, the aminoacrylate formed from L-serine is reported to have a half-life in aqueous media of 1.5 s,[26] which is equivalent to a first-order rate constant of 0.45 s^{-1}. This is consistent with a k_{cat} of 0.8 s^{-1} reported for aminoacrylate hydration produced by a PLP-dependent D-serine dehydratase from chicken kidney.[27] However, this is not consistent with the k_{cat} for pyruvate production of 280 s^{-1} determined for *lp*LSD and is *prima facie* evidence of the enzyme-assisted conversion of aminoacrylate to pyruvate and ammonia in the case of *lp*LSD (Scheme 3). Other studies have also reported evidence that suggests that the conversion of aminoacrylate to pyruvate and ammonia may be enzyme-assisted in some cases. An early paper[28] describing D-serine dehydratase from *E. coli*, a PLP enzyme, concludes that the enzyme reprotonates the bound aminoacrylate with retention of configuration with respect to the original hydroxyl group. Another study of the same enzyme concludes that the final conversion to pyruvate occurs on the enzyme with a k_{cat} of approximately 90 s^{-1}.[29,30] In light of these observations, the production of pyruvate is depicted as occurring on the enzyme in Scheme 3 rather than in solution as is often assumed. Thus, the catalytic mechanism appears to differ in several aspects from many PLP-based dehydratases.

At first glance, the Fe−S cluster-dependent L-serine dehydratases appear to have a surprisingly high K_m for the substrate. The recent evidence that these enzymes may play a role in regulating intracellular L-serine concentration in the context of cell wall synthesis suggests that they must keep L-serine levels relatively low without completely depleting the L-serine pool. The mechanism depicted in Scheme 3 provides a way in which this may be accomplished. The enzymes would display relatively low activity at low serine concentration so as not to completely deplete the serine pool, which is presumably being replenished by some other process, but would display a nonlinear or cooperative increase in activity as serine concentrations increased. This regulation is accomplished by the requirement for substrate to bind to a second, noncatalytic, site that displays a K_d considerably higher than that of the catalytic site. The intracellular serine concentration would not necessarily have to reach or exceed the K_m for the enzyme to be effective in controlling the cellular flux of serine and maintaining optimal levels.

The K_m for L-alanine for the MurC ligase from *E. coli* is 0.05 mM at pH 8.0 and 0.3 mM at pH 7.0,[31] and the K_m for L-serine for the MurC ligase is 1.2 mM at pH 8.0. If the K_m for L-serine follows the same trend as that for L-alanine, the K_m for L-serine would be estimated to be approximately 7−10 mM at pH 7.0. This K_m is in the range of highest activity for *lp*LSD at pH 7.0. Both *E. coli* and *L. pneumophila* are Gram-negative bacteria of the gamma proteobacteria class, and both contain Type 2 L-serine dehydratases that display considerable sequence homology.[1] To the extent that parallels can be drawn between the *E. coli* and *L. pneumophila* enzymes, the activity of *lp*LSD increases sharply in the L-serine concentration range that would effectively interfere with MurC ligase incorporation of L-alanine.

The structural homology of the β domain of *lp*LSD with the ASB domain of *mt*PGDH and the role of the ASB domain as a second substrate binding site strongly suggest that the β domain of *lp*LSD may also be where the second substrate binding site is located. If this turns out to be the case, it implicates a conformational change occurring in the β domain that is translated to the α domain where the catalytic site is found and the only tryptophan residue in the protein resides. Interestingly, that tryptophan is within the first 30 residues of the amino-terminal portion of the α domain and may in fact form part of, or be near, the interface with the β domain. The conformational change, transmitted at the domain interface, would subsequently affect the catalytic site. Thus, the data support a mechanism in which two binding sites for the substrate, one catalytic and one noncatalytic, combine to influence the catalytic turnover of the substrate in a way that is more sensitive to changes in substrate concentration than if binding occurred only at the catalytic site.

The L-serine dehydratases are only the second group of enzymes found to contain an ASB domain, the first consisting of some, but not all, D-3-phosphoglycerate dehydrogenases. In both cases, the ASB domain functions by providing a second, noncatalytic site for the substrate that exerts influence on the catalytic site. It is interesting to note that both enzymes are involved in some aspect of L-serine metabolism. Phosphoglycerate dehydrogenase is involved in L-serine biosynthesis, while L-serine dehydratase is involved in L-serine degradation. Given the relatively rare occurrence of the ASB domain, at least among proteins whose structures have been determined, the question of whether the ASB domain may be unique to enzymes of serine metabolism in bacteria arises. In addition, the Type 1 L-serine dehydratases appear to contain an ASB−ACT domain tandem like that found in phosphoglycerate dehydrogenase from *M. tuberculosis*. This potentially represents another level of control in the Type 1 enzymes that is not found in the Type 2 enzymes represented by *lp*LSD.

Our results provide evidence of a level of continuity in the function of ASB domains and lay the basis for further exploration of specific metabolic control systems in bacteria that have been, for the most part, previously unrecognized.

■ ASSOCIATED CONTENT

Supporting Information

Molecular weight and subunit association of *L. pneumophila* L-serine dehydratase by size exclusion chromatography. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Department of Developmental Biology, Box 8103, Washington University School of Medicine, St. Louis, MO 63110. Phone: (314) 362-3367. Fax: (314) 362-4698. E-mail: ggrant@wustl.edu.

Notes

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■ ABBREVIATIONS

LSD, L-serine dehydratase; PGDH, phosphoglycerate dehydrogenase; ACT, Asp kinase, chorismate mutase, TyrA; ASB, allosteric substrate binding.

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