Holmquist, B., & Vallee, B. L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 6216–6220.
Kam, C. M., Nishino, N., & Powers, J. C. (1979) *Biochemistry 18*, 3032–3038.
Kerr, M. A., & Kenny, A. J. (1974a) *Biochem. J. 137*, 477–488.
Kerr, M. A., & Kenny, A. J. (1974b) *Biochem. J. 137*, 489–495.
Kester, W. R., & Matthews, B. W. (1977a) *Biochemistry 16*, 2506–2516.
Kester, W. R., Matthews, B. W. (1977b) *J. Biol. Chem. 252*, 7704–7710.
Liebman, M. N., & Weinstein, H. (1984) in *Structure & Motion: Membranes, Nucleic Acids and Proteins* (Clementi, G. E., Sarma, R. H., & Sarma, M., Eds.) Adenine Press, New York (in press).
Malfroy, B., Swerts, J. P., Guyon, A., Roques, B. P., & Schwartz, J. C. (1978) *Nature (London) 276*, 523–526.
Matsas, R., Kenny, A. J., & Turner, A. J. (1984) *Biochem. J. 223*, 433–440.
Matthews, B. W., Colman, P. M., Jansonius, J. N., Titani, K., Walsh, K. A., & Neurath, H. (1972) *Nature (London) 238*, 41–43.
Matthews, B. W., Weaver, L. H., & Kester, W. R. (1974) *J. Biol. Chem. 249*, 8039–8044.
Maycock, A. J., DeSousa, D. M., Payne, L. G., ten Broeke, J., Wu, M. T., & Patchett, A. A. (1981) *Biochem. Biophys. Res. Commun. 102*, 963–969.
Monzingo, A. F., & Matthews, B. W. (1984) *Biochemistry 23*, 5724–5729.
Morihara, K., & Tsuzuki, H. (1970) *Eur. J. Biochem. 15*, 374–380.
Mumford, R. A., Pierzchala, P. A., Strauss, A. W., & Zimmerman, M. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6623–6627.
Mumford, R. A., Zimmerman, M., Broeke, J., Taub, D., Joshua, H., Rothrock, J. W., Hirshfield, J. M., Springer, J. P., & Patchett, A. A. (1982) *Biochem. Biophys. Res. Commun. 109*, 1303–1309.
Murthy, L. R., Glick, S. D., Almenoff, J., Wilk, S., & Orlowski, M. (1984) *Eur. J. Pharmacol. 102*, 305–313.
Nishino, N., & Powers, J. C. (1979) *Biochemistry 18*, 4340–4347.
Orlowski, M., & Wilk, S. (1981) *Biochemistry 20*, 4942–4950.
Orlowski, M., Michaud, C., & Chu, T. G. (1983) *Eur. J. Biochem. 135*, 81–88.
Pfleiderer, G. (1970) *Methods Enzymol. 19*, 514–521.
Roques, B. P., Fournie-Zaluski, M. C., Soroca, E., Lecomte, J. M., Malfroy, B., Llorens, C., & Schwartz, J. C. (1980) *Nature (London) 288*, 286–288.
Schwartz, J. C., Malfroy, B., & De La Baume, S. (1981) *Life Sci. 29*, 1715–1740.
Sullivan, S., Akil, H., & Barchas, J. D. (1978) *Commun. Psychopharmacol. 2*, 525–531.
Titani, K., Hermodson, M. A., Ericsson, L. H., Walsh, K. A., & Neurath, H. (1972) *Nature (London) 238*, 35–37.
Warshel, A. (1981) *Acc. Chem. Res. 14*, 284–290.
Weaver, L. H., Kester, W. R., & Matthews, B. W. (1977) *J. Mol. Biol. 114*, 119–132.

Kinetic Studies of L-Aspartase from *Escherichia coli*: Substrate Activation†

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ABSTRACT: The enzyme L-aspartase from *Escherichia coli* was observed to have a time lag during the production of aspartic acid from fumarate and ammonia. This time lag is pH dependent, with little lag observed below pH 7.0 and a very extensive lag observed above pH 8.0. This time lag was also found to be dependent on both substrate and divalent metal ion concentrations and on the degree of proteolysis of L-aspartase. The observed lag, in the reaction examined in the amination direction, has been found to be correlated with the nonlinear kinetics seen at higher pH in the deamination direction. Both phenomena are consistent with a model in which there is a separate activator site for the substrate, L-aspartic acid, that is distinct from the enzyme active site. Occupation of this site by the substrate, or by various substrate analogues, eliminates both the nonlinearity and the time lag. The D isomer of aspartic acid, which does not bind at the active site, can bind at this newly identified activator site.

The enzyme L-aspartase (L-aspartate ammonia-lyase, EC 4.3.1.1) catalyzes the reversible deamination of L-aspartic acid to yield fumaric acid and ammonia. The enzyme from *Escherichia coli* is composed of four apparently identical subunits with a molecular weight of about 48 000. Limited proteolysis of the enzyme with trypsin (Mizuta & Tokushige, 1975), as well as several other proteases (Yumoto et al., 1982), results in a severalfold increase in L-aspartase activity measured at pH 7.0. The divalent metal ion requirements and the circular dichroism and UV spectra of the enzyme are also changed on trypsinolysis (Mizuta & Tokushige, 1976). These changes appear to be concomitant with the loss of one or several small peptides, seven to eight amino acids in length, from the carboxyl-terminal end of the polypeptide chain (Yumoto et al., 1980, 1982).

L-Aspartase displays complex kinetics at higher pH, with positive cooperativity observed at pH 7.5 and above (Williams and Lartigue, 1967; Rudolph & Fromm, 1971). Williams & Scott (1968) have reported that certain nucleotide effectors either activate (AMP, IMP) or inhibit (GTP, UTP) L-as-

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partase from *Bacterium cadaveris* and have suggested a regulatory role for nucleotides with L-aspartase. However, they and others have had difficulty in reproducing these results (Rudolph & Fromm, 1971; W. E. Karsten and R. E. Viola, unpublished results). Since these earlier reports, there has been little additional work aimed at characterizing the nonclassical kinetics exhibited by L-aspartase or at examining the metabolic role and regulatory behavior of this enzyme.

In this paper, we present evidence that is consistent with the non-Michaelis–Menten kinetics that have been reported, as well as our observations of a previously unreported pH-dependent time lag when the enzyme-catalyzed reaction is examined in the direction of aspartic acid production.

Experimental Procedures

Soluble trypsin and Sephadex G-200 were purchased from Sigma Chemical Co. Trypsin lima bean inhibitor was from Millipore Corp. L-Aspartic acid, fumaric acid, and all substrate analogues were from Sigma and were used without further purification. Molecular weight marker proteins were from Pharmacia Fine Chemicals. All other chemicals were reagent grade.

Enzyme Purification. L-Aspartase was purified from *Escherichia coli*, strain B, initially by a modification of the method of Rudolph and Fromm (1971) and subsequently by our recently developed dye–ligand affinity chromatography method (Karsten et al., 1985). The enzyme obtained was approximately 85% pure (Rudolph and Fromm procedure) or apparently homogeneous (Karsten et al. procedure) as judged by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (Weber & Osborn, 1969).

Assay. L-Aspartase activity in the forward or deamination direction was determined spectrophotometrically at 30 °C by measuring the formation of fumarate at 240 nm. Due to the strong absorbance of fumarate at 240 nm, the amination or reverse reaction was followed by measuring the disappearance of fumarate on the high-wavelength shoulder of the absorbance peak. Assays were run on either a Perkin-Elmer Lambda-1 or a Varian DMS-90 recording spectrophotometer equipped with constant-temperature cell housings and a circulating water bath. All assays were run in 1- or 3-mL total volume in 1-cm path-length cuvettes. Standard assay mixtures in the deamination direction contained 50 mM buffer, varying amounts of L-aspartic acid, and sufficient levels of divalent metal ions to ensure saturation of the enzyme based on the metal ion affinities of L-aspartase (C. J. Falzone and R. E. Viola, unpublished observations) and aspartic acid (Khazaeli & Viola, 1984). Typical assays in the amination direction contained fumaric acid, ammonium sulfate, divalent metal ion, and 50 mM buffer. The specific buffer used as well as concentrations of substrate, substrate analogues, and divalent metal ions was as noted in the text. Unless stated otherwise, all reactions were initiated by the addition of 0.1–1.0 μg of enzyme to the assay. All substrate and substrate analogue stock solutions were adjusted before use to approximately the pH of the final assay mixture with either tris(hydroxymethyl)aminomethane (Tris) base or HCl.

Trypsinolysis. Typical trypsinolysis conditions were as follows: To 0.35 mg of L-aspartase in potassium phosphate, pH 7.0, containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 1 mM dithiothreitol (DTT) in a total volume of 0.5 mL was added 2 μg of trypsin. The trypsinolysis reaction was terminated by the addition of 4 μg of trypsin lima bean inhibitor when the L-aspartase activity at pH 7.0 without added metal had dropped below 10% of the activity with added metal (5 mM magnesium acetate).

Gel Filtration. Sephadex G-200 was swelled for 48 h and poured into a 10 × 400 mm column (bed volume 30 mL). The column was flushed and equilibrated with either 50 mM phosphate (pH 7) or Tris (pH 8.9) containing 0.5 mM EDTA and DTT. The column was loaded with 2 mg of L-aspartase in 1 mL of the appropriate buffer. Sucrose was added to the enzyme sample to increase the viscosity and allow the enzyme to load onto the column as a focused band. The column was eluted with the corresponding equilibration buffer, and fractions were analyzed by monitoring the absorbance at 280 nm and by activity assays. Typical recovered activities in these experiments were about 50%.

Data Analysis. For the reactions followed as a function of substrate concentration, velocities were fitted to the equation:

$$v = VA/(K + A) \quad (1)$$

by a computer program that assumed equal variance in the data (Cleland, 1967). Nonlinear plots were fitted in segments.

A lag in a reaction time course can be described (Frieden, 1979) by the equation:

$$v_t = v_f + (v_i - v_f)e^{-kt} \quad (2)$$

where v_i and v_f are the initial and final velocities, respectively, v_t is the velocity at time t, and k is the rate constant for the transition. The lag times for the reaction in the reverse direction were calculated by drawing tangents to the initial and final portions of the reaction time courses to determine v_i and v_f (see Figure 1). The relaxation time for the transition from the initial to the final velocity is the point of intersection of these tangents. The reciprocal of this relaxation time is the apparent rate constant for the transition.

Results

A preliminary communication on the properties of L-aspartase that had been activated by trypsin treatment (Mizuta & Tokushige, 1975) reported little change in the kinetic parameters from those of the nonproteolyzed enzyme. A detailed kinetic analysis of L-aspartase from *E. coli* was begun to determine the possible effects of proteolysis on the kinetic mechanism of this enzyme. During the kinetic characterization of partially trypsinized L-aspartase, it was observed that when the enzyme was assayed in the direction of aspartic acid production at neutral pH, a time lag occurred before the final steady-state rate was attained. A time lag was also observed during subsequent studies with the native enzyme (Figure 1). Further investigation revealed that the time lag for the trypsinized enzyme was pH dependent, with little or no lag observed at about pH 6.5 and below, while the relaxation time for attainment of the final steady-state rate became increasingly longer at higher pH (Figure 2). This lag was also found to be dependent on both substrate and divalent metal ion concentrations. Lower levels of fumarate, NH_4^+, or divalent metal ions resulted in longer lag times before the time course of the reaction became linear. Higher substrate and metal ion concentrations tended to shorten lag times. Saturating levels of certain alkali earth metal ions, such as Mg^{2+} and Ca^{2+}, will decrease the lag times, while saturating levels of certain transition metal ions, such as Mn^{2+} and Co^{2+}, appear to completely eliminate the lag under the conditions observed. Under certain conditions, the observed lag time became quite extensive. At higher pH, with low concentrations of fumarate, NH_4^+, and divalent metal ions, a lag time as long as 3 h has been observed before a linear, steady-state rate was attained.

Subsequent work revealed that the lag time observed with the unproteolyzed (native) form of L-aspartase had similar properties to the lag shown by the proteolyzed enzyme. The

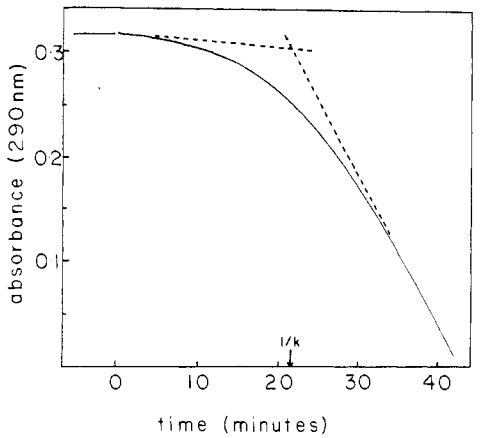

FIGURE 1: Time course of the L-aspartase-catalyzed reaction. The reaction was followed at pH 8.4 in the amination direction as described under Experimental Procedures. The assay mixture contained 100 mM Tris-HCl, 10 mM fumaric acid, 2 mM $MgCl_2$, and 50 mM ammonium sulfate. The tangents drawn to the initial and final segments of the time course are used for calculation of the relaxation time for the transition.

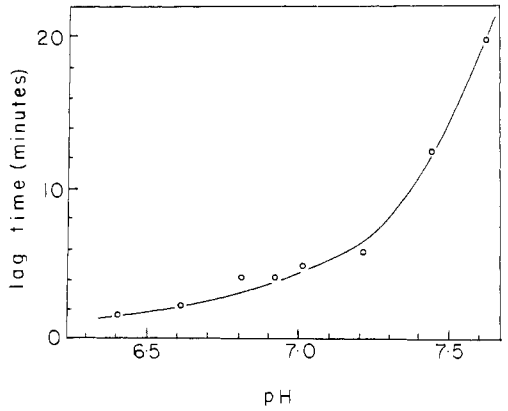

FIGURE 2: pH dependence of the L-aspartase time lag. The time lag was measured as the relaxation time in going from the initial to the final steady-state rate. Assay conditions were the same as in Figure 1, except that the buffer was changed to 100 mM Tris–HEPES.

pH dependence of the lag displayed by the native enzyme was, however, somewhat different. The native enzyme showed a significant lag at about pH 8.0 and above, as compared to the lag observed at about pH 7.0 and above for the partially trypsinized enzyme. Similar to the trypsinized enzyme, the lag time displayed by native L-aspartase became progressively longer at higher pH. The final steady-state rate was observed to be linearly dependent on enzyme concentration when examined over a 50-fold enzyme concentration range.

Several hypotheses were examined that could explain this nonlinear kinetic behavior. The possibility that L-aspartase exists as dissociated inactive monomers that slowly reassociate during the course of the reverse reaction was investigated by gel filtration chromatography. Samples of enzyme were applied to Sephadex G-200 columns that had been equilibrated at pH 7.0 or 8.9. L-Aspartase eluted as a single peak at the molecular weight of the tetramer, with no indication of a protein peak at the molecular weight of the monomer at either pH value.

Since the initial lag time studies were conducted with relatively crude enzyme preparations with specific activities (units per milligram of total protein) in the range of 2–8 (3–10% pure enzyme), the possibility was considered that an inhibitor might be present in these crude enzyme preparations. This inhibitor might then slowly dissociate on dilution of the enzyme into

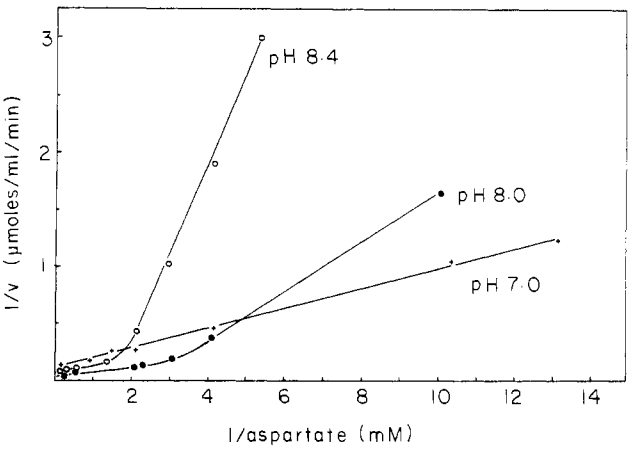

FIGURE 3: Reciprocal plot of L-aspartase activity at several pH values. Assays with native enzyme were conducted as described under Experimental Procedures with the reaction mixture containing 50 mM buffer (HEPES, pH 7 and 8; Tris, pH 8.4), 5 mM Mg^{2+}, and the indicated concentrations of L-aspartic acid.

the assay mixture, thus freeing the enzyme from inhibition. An attempt was made to isolate and identify this putative inhibitor by gel filtration, and by subsequently adding back eluted fractions to fractions containing L-aspartase activity and checking for any effects on the extent of nonlinearity. Also, crude enzyme preparations were mixed with significantly purer preparations of enzyme, and the effects on the nonlinearity were determined. No evidence for the presence of an inhibitor was found in these experiments. In addition, unless the postulated inhibitor is very tightly bound, which is not indicated since any inhibitor must presumably dissociate during a reverse reaction assay, then it is reasonable to assume that simply purifying L-aspartase to homogeneity should result in the loss of nonlinear kinetics. However, subsequent work showed that apparently homogeneous enzyme, as judged by SDS–polyacrylamide gel electrophoresis, also displays a significant lag time under a variety of conditions.

Another explanation which was considered for the lag time was a slow conformational change occurring during the course of the reverse reaction, from an inactive to an active form of L-aspartase. To test this idea, several preincubation experiments were conducted in which either fumarate, ammonia, or divalent metal ions were eliminated from a preincubation mixture. Addition of the missing component to initiate the reaction resulted in a nonlinear time course, with the observed lag essentially identical with control assays.

During product inhibition studies that were being conducted, it was observed that the addition of low concentrations of L-aspartate would eliminate the time lag. If L-aspartate (at about 1 mM) was present initially in a reverse reaction assay, no lag was detected, and the initial rate observed was identical with the final linear steady-state rate. Also, addition of L-aspartate during the lag phase of a reverse reaction assay resulted in an immediate activation to the maximum steady-state rate.

It has been known that in the forward (deamination) direction L-aspartase displays linear kinetics at pH 7.0 but nonlinear kinetics at about pH 7.5 and above (Williams & Lartigue, 1967). As can be seen in Figure 3, reciprocal plots of the L-aspartase forward reaction activity depart from linearity at progressively higher L-aspartate concentrations at higher pH. Partially trypsinized L-aspartase also displays nonlinear kinetics, but these deviations from linearity begin at lower pH values than the native enzyme. A time lag is seen in the reverse direction with native or proteolyzed enzyme only

Table I: Relationship of Nonlinear Reciprocal Plots to the pH-Dependent Lag Time[a]

enzyme form	pH	concn of L-aspartic acid (mM)	
		deamination reaction[b]	amination reaction[c]
native	7.0	linear	no lag
	8.0	0.35	0.2
	8.4	0.62	0.5
partially proteolyzed	6.3	linear	no lag
	7.0	0.33	0.37
	8.4	3.8	3.6

[a] Enzyme assays were conducted in the presence of 5 mM Mg^{2+} and 50 mM buffer [2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.3, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.0 and 8.0, and Tris, pH 8.4]. [b] Concentration of aspartic acid at the point where the reciprocal plot deviates from linearity. [c] Concentration of aspartic acid produced at the point where the reaction time course becomes linear.

at pH values where nonlinear kinetics are seen in the forward direction. Additionally, at pH values where linear kinetics are observed, no time lag is detected in the reverse direction. The correlation between the nonlinear kinetics and the lag time can also be shown to be a quantitative relationship. As can be seen in Table I, under similar conditions there is a strong relationship between the amount of aspartic acid produced in the reverse reaction at the point that the time course becomes linear and the substrate concentration below which the reciprocal plots begin to depart from linearity. This correlation remains true under a variety of pH and divalent metal ion conditions. At high pH, where the lag time is longer, more aspartic acid is required to be produced in the reverse direction before a maximal steady-state rate can be achieved. With the same conditions of pH, divalent metal ion, and substrate concentration, partially trypsinized enzyme displays a longer lag than the native enzyme. Examination of Table I reveals that under similar conditions more aspartic acid was produced before the lag was fully eliminated with partially trypsinized L-aspartase as compared to the native enzyme.

Since the concentration of the substrate L-aspartate appears to play a significant role in these deviations from Michaelis–Menten kinetics, the effects of several aspartic acid analogues on the nonlinear kinetics were examined. Kinetic studies have shown that D-aspartic acid does not bind at the active site of L-aspartase (R. E. Viola and R. B. Gates, unpublished results). No enzymatic activity is observed with D-aspartic acid, and at concentrations as high as 75 mM, the D isomer does not inhibit the steady-state rate of the L-aspartase-catalyzed reaction. However, relatively low concentrations of D-aspartic acid (2–5 mM) do eliminate much of the nonlinear kinetics displayed by native L-aspartase at pH 8.4 (Figure 4). At somewhat higher concentrations (about 15 mM), the D-isomer totally eliminates the nonlinear kinetics. Also, under similar conditions, the presence of D-aspartic acid shortens or eliminates the lag time in the reverse direction. The presence of several other aspartic acid analogues that were determined to be poor or noninhibitors of L-aspartase could, at relatively low concentrations (1–20 mM), also eliminate the nonlinear kinetics in the forward direction and the time lag in the reverse direction. These analogues included α-methylaspartic acid, L-glutamate, D-alanine, and *N*-formylaspartic acid. Conversely, several other noninhibitory aspartic acid analogues including L-alanine, L-serine, L-cysteic acid, and L-asparagine gave no indication, at concentrations up to 10 mM, of the ability to affect the nonlinear kinetics or the time lag of the native enzyme at pH 8.4.

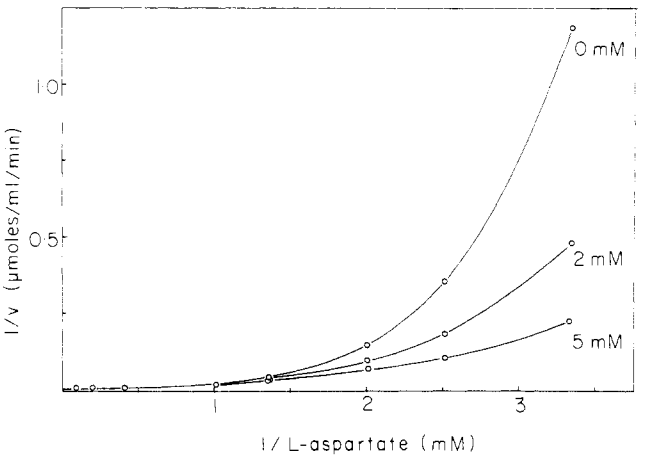

FIGURE 4: Effect of D-aspartic acid on the nonlinear reciprocal plots of L-aspartase. Assays with native enzyme were conducted at pH 8.4 under the same conditions described in Figure 3, except for the addition of varying amounts of D-aspartic acid.

DISCUSSION

Models have previously been proposed (Monod et al., 1965; Koshland et al., 1966) to account for the cooperative kinetics and the effects of modifiers on allosteric enzymes. With the observation of time-dependent changes in enzyme activities, these models have been extended to allow for the possibility of slow binding of substrates or modifiers and/or conformational changes to account for the transient kinetics (Ainslie et al., 1972).

The observation of a pH-dependent time lag during the amination of fumaric acid catalyzed by L-aspartase has led to several experiments in an attempt to provide an explanation for this phenomenon. The gel filtration molecular weight experiments indicated that no significant dissociation of the tetramer had occurred under the pH conditions in which this time lag was observed. These experiments were, however, carried out at higher protein concentrations than are normally used in the kinetic studies. These results are consistent with the Sepharose chromatography data of Mizuta and Tokushige (1976) utilizing trypsinized enzyme, and with the Sephadex molecular weight experiments and ultracentrifugation experiments of Rudolph and Fromm (1971) conducted at pH 7.0 and 9.0 with native L-aspartase at low protein concentrations. Various experiments also failed to support the existence of a dissociable inhibitory factor or a slow conformational change from an inactive to an active enzyme form induced by the presence of substrates or divalent metal ion.

A hypothesis that was developed during the course of this investigation has been found to be consistent with the available experimental evidence. The data support the idea that there is an allosteric activator site on the enzyme to which the substrate L-aspartic acid binds. In the absence of L-aspartic acid, the enzyme L-aspartase exists in a relatively inactive form at neutral pH and above.[1] When the enzyme is incubated in an assay mixture with fumaric acid, ammonia, and a divalent metal ion, there is no L-aspartic acid present initially. Consequently, L-aspartase would turn over very slowly, producing L-aspartic acid at a minimal rate. However, as the concentration of L-aspartic acid slowly increases, this product will bind to the activator site on the enzyme, shifting the equilibrium to the more active enzyme form. Thus, as the concentration of L-aspartic acid builds up, the reaction rate

[1] The enzyme may be totally inactive in the absence of aspartic acid but shows some residual activity because of the presence of low levels of bound aspartic acid in the purified enzyme.

would increase in a highly cooperative manner until a linear steady-state rate is reached at the point that the activator site becomes fully occupied.

This hypothesis does not require the slow binding of L-aspartate or any slow protein conformational changes to account for the observed transient kinetics. The slow production of the product by the enzyme is sufficient to explain the observed time-dependent changes. No lag would be observed in the deamination direction under normal conditions since L-aspartic acid would already be present to occupy the activator site.

There are several lines of evidence that support this hypothesis. The nonlinear kinetics displayed by L-aspartase at high pH in the forward direction are indicative of the interaction of more than one molecule of L-aspartic acid with the enzyme. Significantly, the nonlinear kinetics are seen only under pH and divalent metal ion conditions during which the time lag is observed in the reverse direction. Under conditions in which linear kinetics are seen in the deamination direction, no time lag is observed in the amination direction. A strong correlation also exists between the amount of aspartic acid produced at the point that the reverse direction time course becomes linear and the amount of aspartic acid produced at the point where the reciprocal plots depart from linearity. This correlation is observed under varying conditions of pH and the state of proteolysis of the enzyme. Depending on the concentration, the initial presence of L-aspartic acid in a reverse direction assay will either shorten or totally eliminate the observed lag. In these cases, the minimum levels of L-aspartic acid required to eliminate the lag under a given set of conditions were also consistent with the data in Table I. In addition, consistent with the observation that the lag is longer at higher pH values, the amount of aspartic acid required to achieve a maximal steady state rate in the reverse direction also increases as the pH increases. Clearly, the more L-aspartic acid that must be produced to fully occupy the proposed activator site, the longer the observed lag time will be and the more nonlinear the reciprocal plots will be in the forward reaction.

It would be premature to attempt to ascribe any physiological significance to the substrate activation observed for the isolated and purified enzyme. While the relative affinity of L-aspartic acid for this activator site is certainly comparable to its affinity for the active site, the effects of pH, metal ion, and substrate concentrations, and the degree of proteolysis of L-aspartase, must first be examined in greater detail.

Evidence that the activator site is distinct from the binding events occurring at the active site comes from the series of experiments with D-aspartic acid. At concentrations as high as 75 mM, D-aspartic acid does not inhibit the L-aspartase-catalyzed reaction, indicating that D-aspartic acid has low affinity for the active site. However, relatively low levels of the D isomer eliminate the nonlinear kinetics displayed by the enzyme, as well as eliminating the lag time in the reverse direction in a manner similar to L-aspartic acid. High ionic strength showed no significant activating effect or capacity to eliminate the lag time, indicating that the D-aspartate effects were not the result of any general, nonspecific changes in ionic strength. D-Aspartic acid must be interacting with L-aspartase at some site distinct from the active site. Since several other analogues of aspartic acid display effects similar to D-aspartate, while other analogues did not, this indicates a rather unique specificity to the activator site.

Added in Proof

After the manuscript was submitted, a paper reporting the activation of aspartase by L-aspartic acid was published (Ida & Tokushige, 1985).

Acknowledgments

Some preliminary experiments on this project were conducted while the authors were in the Department of Chemistry, Southern Illinois University.

References

Ainslie, G. R., Jr., Shill, J. P., & Neet, K. E. (1972) *J. Biol. Chem. 247*, 7088.

Cleland, W. W. (1967) *Adv. Enzymol. Relat. Areas Mol. Biol. 29*, 1.

Frieden, C. (1979) *Annu. Rev. Biochem. 48*, 471.

Ida, N., & Tokushige, M. (1985) *J. Biochem. (Tokyo) 98*, 35.

Karsten, W. E., Hunsley, J. R., & Viola, R. E. (1985) *Anal. Biochem. 147*, 336.

Khazaeli, S., & Viola, R. E. (1984) *J. Inorg. Biochem. 22*, 33.

Koshland, D. E., Jr., Nemethy, G., & Filmer, D. (1966) *Biochemistry 5*, 365.

Mizuta, K., & Tokushige, M. (1975) *Biochem. Biophys. Res. Commun. 67*, 741.

Mizuta, K., & Tokushige, M. (1976) *Biochim. Biophys. Acta 452*, 253.

Monod, J., Wyman, J., & Changeux, J. P. (1965) *J. Mol. Biol. 12*, 88.

Rudolph, F. B., & Fromm, H. J. (1971) *Arch. Biochem. Biophys. 147*, 92.

Weber, K., & Osborne, M. (1969) *J. Biol. Chem. 244*, 4406.

Williams, V. R., & Lartigue, D. J. (1967) *J. Biol. Chem. 242*, 2973.

Williams, V. R., & Scott, R. M. (1968) *Biochem. Biophys. Res. Commun. 31*, 433.

Yumoto, N., Tokushige, M., & Hayashi, R. (1980) *Biochim. Biophys. Acta 616*, 319.

Yumoto, N., Mizuta, K., Tokushige, M., & Hayashi, R. (1982) *Physiol. Chem. Phys. 14*, 391.