Kinetic Mechanism of Serine Transacetylase from *Salmonella typhimurium*†

Lee-Shan Leu and Paul F. Cook*

Department of Microbiology and Immunology, University of North Texas Health Science Center at Forth Worth, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107

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ABSTRACT: Serine transacetylase from *Salmonella typhimurium* was purified to near homogeneity. A complete initial velocity study in both reaction directions suggests that the enzyme catalyzes the conversion of acetyl CoA and L-serine to *O*-acetyl-L-serine (OAS) and coenzyme A (CoASH) by a ping pong bi bi kinetic mechanism. Initial velocity patterns in the absence of added inhibitors in both directions are best described by a series of parallel lines. Product inhibition by OAS is competitive with respect to acetyl CoA and noncompetitive with respect to L-serine, while product inhibition by L-serine is competitive against CoASH and noncompetitive against OAS. Glycine and *S*-methyl-L-cysteine (SMC) were used as dead-end analogs of L-serine and OAS, respectively. Glycine is competitive against L-serine, and uncompetitive against acetyl CoA, while SMC is competitive against OAS and uncompetitive against CoASH. All of the above inhibition patterns are consistent with those predicted for a single site ping pong bi bi kinetic mechanism. The equilibrium constant for the transacetylase reaction is 15 in the direction of serine acetylation. The constant was measured by monitoring the change in CoASH concentration obtained for reactions in which the ratio of acetyl CoA/CoASH was constant and the ratio of OAS/serine was varied. The K_{eq} calculated from the Haldane relationship is in good agreement with the value obtained by direct measurement.

The biosynthesis of L-cysteine in *Salmonella typhimurium* proceed via a two-step enzymatic pathway (Kredich & Tomkins, 1966). Serine transacetylase (STA)[1] is responsible for the conversion of acetyl CoA and L-serine to OAS and CoASH. The second step is catalyzed by OASS, which converts OAS and sulfide to L-cysteine and acetate.

A physical association of STA with 5% of the total cellular OASS has been reported by Kredich et al. (1969). These investigators also reported that OAS at a concentration of 0.1–1 mM causes this complex to dissociate into one molecule of STA (M_r, 160 000) and two molecules of OASS (M_r, 68 000). The complex, cysteine synthetase, appears to be feedback inhibited at the level of STA by the product L-cysteine (Kredich et al., 1969). The multienzyme complex does not channel the intermediate product OAS, between the active sites of the two component enzymes. Rather, OAS is released into solution and must reassociate with OASS to be converted to L-cysteine (Cook & Wedding, 1977). The existence of a complex between OASS and STA is thus interesting and to determine the reason for this phenomenon is the long-range goal of this research.

Kredich et al. (1969) initially resolved STA from the cysteine synthetase multienzyme complex which was purified by conventional means. More recently, STA has been resolved from the complex after being adsorbed to an anti-OASS IgG column and eluted with 50 mM OAS (Baecker & Wedding, 1980). The final specific activity of the STA is 400 units/mg, giving a turnover number of about 200 s^{-1}. Apparent K_m values for acetyl CoA (0.1 mM) and L-serine (0.7 mM) have been measured. L-Cysteine is reported to feedback inhibit the resolved STA and is noncompetitive vs L-serine, but competitive vs acetyl CoA with a K_i of 1 μM (Kredich et al., 1969). Very little is presently known concerning the kinetic and chemical mechanisms of acyltransferases that use an alcohol as an acyl group acceptor, and virtually nothing is known of the mechanism of regulation of STA and related acyltransferases. This report provides information on the kinetic mechanism of STA.

MATERIALS AND METHODS

Chemicals. L-Serine, L-cysteine, L-alanine, L-aspartate, *O*-acetyl-L-serine, glycine, acetyl CoA, DTNB, ammonium sulfate, and streptomycin sulfate were from Sigma. Citric acid and sodium ammonium phosphate were from Fisher. Acrylamide and bisacrylamide for PAGE were from Bio-Rad, while Hepes was from Research Organics, Inc. All other chemicals were obtained from commerical sources and were reagent grade.

Enzymes. A Cys^- strain of *S. typhimurium*, DW18.1, transformed with plasmid pRSM41 that contains the gene for ampicillin resistance and the *cys E* gene that encode STA was the generous gift of Dr. N. M. Kredich, Duke University Medical Center. Cells were grown 7 h on a shaker in 2-L Erlenmyers at 37 °C, 300 rpm in Vogel-Bonner medium E with 0.02% (w/v) magnesium sulfate as the sulfur source in the presence of 75 μg/mL ampicillin. Cells were harvested by centrifugation at 6300*g* for 15 min at 4 °C. About 4 g of wet cell paste was obtained per liter of VBE medium.

The first three steps of the purification procedure are the same as those used in purification of OASS-A (Hara et al., 1990) and are a modification of the original method of Becker et al. (1969). The majority of the STA activity precipitates between 30% and 50% ammonium sulfate saturation. The ammonium sulfate pellet is redissolved in 10 mM Hepes, pH 8 (buffer A) and heated to 60 °C as described by Becker et al. (1969). The supernatant obtained from the heat treatment was then dialyzed against 1.5 L of buffer A for 20 h at 4 °C.

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[1] Abbreviations: STA, serine transacetylase; OAS, *O*-acetyl-L-serine; OASS, *O*-acetylserine sulfhydrylase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; TNB, 5-thio-2-nitrobenzoate; CoASH, coenzyme A; SMC; *S*-methyl-L-cysteine.

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Table 1: Purification of Serine Transacetylase from *S. typhimurium*

purification step	volume (mL)	protein (OD_{280}/mL)[a]	enzyme units/mL	enzyme total unit (×10³)	specific activity (units/OD_{280})[a]	*n*-fold purification	yield (%)
crude extract	119	160	88	10.5	.55	1	100
streptomycin sulfate	141	96	71	10.0	.74	1.3	95
ammonium sulfate	121	25.3	73	8.9	2.9	5.3	85
heat treatment	96	20.7	77	7.4	3.7	6.7	70
DEAE-5PW	23	6.4	218	5.0	34	62	48
dye matrix blue A	18	2.5	165	3.0	66	120	28

[a] A value of 1.0 OD_{280} unit per mg of protein was assumed according to Kredich et al. (1969).

The dialysate was applied to a DEAE-5PW HPLC column (2.15 × 15 cm) equilibrated with buffer A. The chromatogram was developed using a 0 to 0.5 M NaCl gradient in buffer A in 125 min. Serine transacetylase elutes in the 280–380 mM NaCl fractions. The major STA-containing fractions (280–310 mM NaCl) were pooled and concentrated using a Filtron Omegacell device. The resulting STA was loaded onto a Dye-matrex blue A column (2.5 × 15 cm). The chromatogram was developed using a 0 to 2.0 M NaCl gradient in 10 mM Hepes, pH 6.5, at a flow rate of 2 mL/min. Those fractions with STA activity were pooled and concentrated to 18 mL using a Filtron Omegacell device. A SDS–PAGE of this purified STA sample showed two adjacent bands which have an apparent molecular weight of 40 000 and a purity of >80%. In order to increase stability, STA was maintained at 4 °C in the presence of 0.5 M NaCl to minimize denaturation of the enzyme.

Enzyme Assays and Initial Velocity Studies. A unit of STA is defined as the amount of enzyme required to produce 1 μmol of 5-thio-2-nitrobenzoate (TNB) in 1 min at pH 7.6 and 25 °C. Reactions were carried out in cuvettes of 1-cm pathlength in a final volume of 1 mL which contained the following: Tris-HCl, pH 7.6, 100 mM; DTNB, 0.5 mM; acetyl CoA, 0.1 mM; L-serine, 5 mM; and an appropriate amount of the enzyme. In the direction of OAS formation, STA was assayed spectrophotometrically by coupling the production of CoASH to the production of TNB via a disulfide exchange reaction with DTNB (Cook & Wedding, 1977). Rates were calculated using an extinction coefficient for TNB at 412 nm of 13 600 M^{-1} cm^{-1}. For the initial velocity measurements in the presence of L-cysteine, STA was assayed at pH 7, 100 mM phosphate buffer in the direction of serine acetylation using the disappearance of absorbance at 232 nm reflecting the thioester bond. Phosphate buffer was used to eliminate the absorbance of Hepes at 232 nm. In the direction of CoASH acetylation, STA was assayed spectrophotometrically at pH 7, 100 mM phosphate buffer by measuring the appearance of absorbance at 232 nm with a 0.1-cm pathlength cuvette. Phosphate at the concentration used does produce a small amount of inhibition. For the latter two cases, a 0.1-cm pathlength cuvette and $\Delta\epsilon_{232}$ of 4500 M^{-1} cm^{-1} were used.

For the initial velocity studies of STA, reactions were initiated by the addition of enzyme and the time course was monitored continuously with a Gilford Model 2600 spectrophotometer equipped with a Hewlett Packard 7225B flatbed plotter. The temperature was maintained at 25 °C using a circulating water bath to heat the thermospacers of the cuvette compartment. A plot of steady-state velocity vs STA concentration is linear over the concentration range used for initial velocity studies and passes through the origin. Initial velocity patterns were obtained by varying one reactant over a range of concentrations less than K_m to 5-fold greater than K_m whenever possible. Product and dead-end inhibition patterns were obtained by measuring the initial velocity at variable concentrations of one reactant at different fixed levels of the second, or at a single fixed level of the second and different fixed concentrations of the inhibitor including zero.

A plate reader assay was developed for the purification procedure in order to assay all of the fractions from a column chromatogram simultaneously. The assay included the following: Hepes, pH 7.0, 100 mM; DTNB, 100 μM; acetyl CoA, 100 μM; L-serine, 5 mM; and an aliquot of the column fraction. The mixtures (minus serine) were preincubated and the reactions were started by adding L-serine.

Data Processing. Reciprocal initial velocities were plotted as a function of reciprocal substrate concentrations. Data were fitted using the appropriate rate equations and the Fortran programs of Cleland (1979). Data conforming to a ping pong mechanism were fitted using eq 1. Data for linear competitive, uncompetitive, and noncompetitive inhibition were fitted using eqs 2–4, respectively. In eqs 1–4, v and V represent initial and

$$v = VAB/(K_aB + K_bA + AB) \quad (1)$$

$$v = VA/[K_a(1 + I/K_{is}) + A] \quad (2)$$

$$v = VA/[K_a + A(1 + I/K_{ii})] \quad (3)$$

$$v = VA/[K_a(1 + I/K_{is}) + A(1 + I/K_{ii})] \quad (4)$$

maximum velocities, respectively; K_a and K_b are K_m values for A and B, respectively; K_{is} and K_{ii} are slope and intercept inhibition constants, while A, B, and I represent reactant and inhibitor concentrations, respectively.

RESULTS

Purification of Serine Transacetylase. Ease of purification of STA has been made possible by its overexpression in a cysteine auxotrophic strain of *S. typhimurium*, DW18.1, which has a mutated *cys* E gene complemented with the *cys* E containing plasmid, pRSM41. Table 1 summarizes the results of the purification procedure. From 21 g of cell paste, approximately 45 mg of STA is obtained with a final specific activity of about 70 units/mg (assayed with nonsaturating concentrations of substrate). Compared to wild-type cells, the STA content is increased ca. 100-fold. Most of the STA precipitates between 30% and 50% ammonium sulfate saturation. The latter procedure removes some of the *O*-acetylserine sulfhydrylase which precipitates between 40% and 70% ammonium sulfate. An overnight dialysis at 4 °C diminishes STA activity by about 30%, but it was found that when the salt concentration is 0.5 M or higher, the STA activity is stable. The purified STA sample reveals two closely spaced bands via SDS–PAGE with apparent molecular weights of ~40 000 and these two bands are also observed in native gels. The two bands were excised from a native gel and activity was determined. Both of the bands are serine transacetylase based on activity measured according to the DTNB assay. The same SDS–PAGE pattern was obtained for STA eluted from

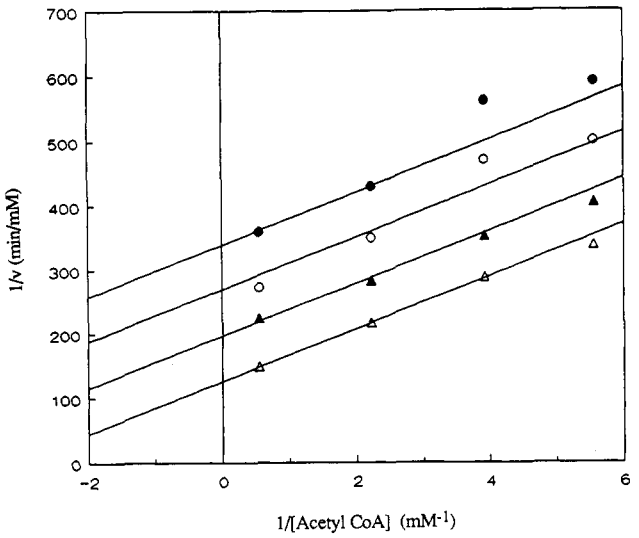


FIGURE 1: Initial velocity patterns of serine acetylation at varying [acetyl CoA] and at [L-serine] of 1.4 (●), 2.0 (○), 3.5 (▲), and 14 (△) mM. Assays were carried out in 100 mM Hepes, pH 7, and 25 °C, and in the presence of 10 mM glycine. Points are experimental and the solid lines are from a fit using eq 1. A STA concentration of 29 ng was used for each assay.

a brown dye affinity column. The addition of a cocktail of protease inhibitors including EDTA, EGTA, PMSF, leupeptin, and pepstatin during the purification procedure did not result in eliminating either of the two SDS–PAGE bands. The purified STA shows no OASS activity.

Effect of Glycine/Alanine. Reactions measured using the DTNB assay exhibit distinct downward curvature over the entire time course of the assay in which no more than 5% of the original acetyl CoA is utilized. Addition of 10 mM glycine or 30 mM L-alanine to the reaction mixture results in a longer linear time course. In order to more accurately measure initial velocities, kinetic studies of serine transacetylase in the both reaction directions were carried out in the presence of 10 mM glycine.

Initial Velocity Studies in the Absence of Products. The time course for STA-catalyzed transacetylation of L-serine exhibited a prominent lag phase prior to attaining the steady state when 0.1 mM DTNB is used in the assay. The lag phase is eliminated by maintaining the concentration of DTNB at 0.45 mM. Initial velocities were calculated on the basis of the initial linear portion of the time courses. Figure 1 shows a double reciprocal plot in the direction of serine acetylation. A family of parallel lines is obtained, suggestive of a ping pong mechanism. An identical pattern is obtained for the reaction in the direction of CoASH acetylation. Kinetic parameters for both cases are summarized in Table 2.

Product Inhibition Studies. In the direction of L-serine acetylation, OAS was tested as a product inhibitor against the two possible substrates. Inhibition against acetyl CoA by OAS is linear competitive with L-serine maintained at a nonsaturating concentration. A qualitatively similar pattern was obtained with L-serine maintained saturating. When the concentration of L-serine is varied and OAS is the inhibitor, linear noncompetitive inhibition is observed. In the direction of CoASH acetylation, L-serine inhibits noncompetitively with respect to OAS and competitively with respect to CoASH. Data from product inhibition studies are summarized in Table 3.

Dead-End Inhibition. In addition to increasing the extent of the STA reaction, glycine is a dead-end analog of serine. In the direction of L-serine acetylation, glycine is competitive vs L-serine at nonsaturating acetyl CoA, while it is uncompetitive vs acetyl CoA at nonsaturating L-serine (Figure 2).

S-Methyl-L-cysteine was used as a dead-end analog of OAS in the reverse reaction direction. *S*-Methyl-L-cysteine is competitive vs OAS at nonsaturating CoASH and is uncompetitive vs CoASH at nonsaturating OAS. Results of dead-end inhibition data are summarized in Table 3.

Equilibrium Constant. The equilibrium constant for the STA reaction is expected to be pH independent since there is no net production or uptake of protons during the course of the transacetylation. The time to reach equilibrium was first established by measuring the change in CoASH concentration with time after addition of STA to solutions similar to those described below. Aliquots were taken as a function of time, STA was removed by ultrafiltration using a Centricon, and the CoASH concentration was measured using DTNB. The reaction was complete in 10 min in all cases. The K_{eq} was measured at pH 6 in 100 mM Mes by fixing the ratio of [acetyl CoA]/[[CoASH] at 1 and varying the ratio of [OAS]/[L-serine] from 0.1 to 20. The reactions were then carried out at varied [OAS]/[L-serine] ratios, filtering 10 min after addition of 0.1 units of STA. A plot of the change in CoASH concentration vs [L-serine] is shown in Figure 3. The point at which the curve crosses the abscissa is equal to the equilibrium constant; K_{eq} = 15.

Table 2: Kinetic Parameters for Serine Transacetylase from *S. typhimurium*

Serine Acetylation[a,b]	
V/E_t	135 ± 5 s^{-1}
$K_{acetyl\ CoA}$	0.40 ± 0.03 mM
$K_{L\text{-serine}}$	3.2 ± 0.2 mM[d]
$V/K_{acetyl\ CoA}E_t$	(3.4 ± 0.1) × 10^5 M^{-1} s^{-1}
$V/K_{L\text{-serine}}E_t$	(4.2 ± 0.2) × 10^4 M^{-1} s^{-1}
CoASH Acetylation[a,c]	
V/E_t	200 ± 10 s^{-1}
K_{CoASH}	1.7 ± 0.2 mM
K_{OAS}	68 ± 9 mM[d]
$V/K_{CoASH}E_t$	(1.2 ± 0.1) × 10^5 M^{-1} s^{-1}
$V/K_{OAS}E_t$	(3.0 ± 0.2) × 10^3 M^{-1} s^{-1}

[a] From a fit of the data using eq 1. [b] The reactions were carried out at pH 7, 100 mM Hepes, and 25 °C. [c] The reactions were carried out at pH 7, 100 mM potassium phosphate, and 25 °C. [d] The K_m values corrected for the presence of glycine are as follows: $K_{L\text{-serine}}$, 1.4 ± 0.1 mM; K_{OAS}, 30 ± 4 mM.

DISCUSSION

Initial Velocity Patterns in the Presence of Glycine. Curvature in the time course of the STA reaction made it difficult to determine the initial velocity. The latter was judged to be a result of severe product inhibition by CoASH. Screening for dead-end inhibitors occasioned the use of L-alanine and glycine as potential analogs of L-serine. Surprisingly, the time courses in the presence of 30 mM L-alanine or 10 mM glycine are linear. This may indicate that there is either a second allosteric site for combination of an amino acid to cause activation, or that combination of an amino acid to the L-cysteine allosteric inhibitory site results in activation. It is also possible that glycine and alanine combine at the active site to cause activation. Activation may be a result of increasing the off-rate of one of the products. This aspect is now under active investigation. The initial velocity patterns obtained in both reaction directions in the absence or presence of 10 mM glycine exhibit a series of parallel lines. The K_m values for L-serine and OAS were corrected for the presence of glycine. The latter behavior is suggestive of a ping pong kinetic mechanism for serine transacetylase (eq 5).

Table 3: Product and Dead-End Inhibition Patterns for Serine Transacetylase from *S. typhimurium*

varied substrate	fixed substrate	inhibitor	pattern[c]	K_{is} ± SE (mM)	K_{ii} ± SE[d] (mM)
		Serine Acetylation[a]			
acetyl CoA	L-serine (3.9 mM)	OAS	C	63 ± 4	
acetyl CoA	L-serine (60 mM)	OAS	C	120 ± 20	
L-serine	acetyl CoA (0.25 mM)	OAS	NC	170 ± 40	180 ± 20
L-serine	acetyl CoA (0.30 mM)	glycine	C	8.3 ± 1.0	
acetyl CoA	L-serine (1.5 mM)	glycine	UC		10.3 ± 0.9
		CoASH Acetylation[b]			
CoASH	OAS (50 mM)	L-serine	C	2.8 ± 0.2	
OAS	CoASH (0.5 mM)	L-serine	NC	3.3 ± 0.4	67 ± 20
OAS	CoASH (1.2 mM)	SMC	C	9.6 ± 1.4	
CoASH	OAS (120 mM)	SMC	UC		24 ± 4

[a] Data were attained at pH 7, 100 mM Hepes, and 25 °C. [b] Data were attained at pH 7, 100 mM potassium phosphate, and 25 °C. [c] C, UC, and NC represent competitive, uncompetitive, and noncompetitive inhibition, respectively. [d] K_{is} and K_{ii} values were determined from fits of the data using eqs 2–4 for competitive, uncompetitive, and noncompetitive inhibition, respectively.

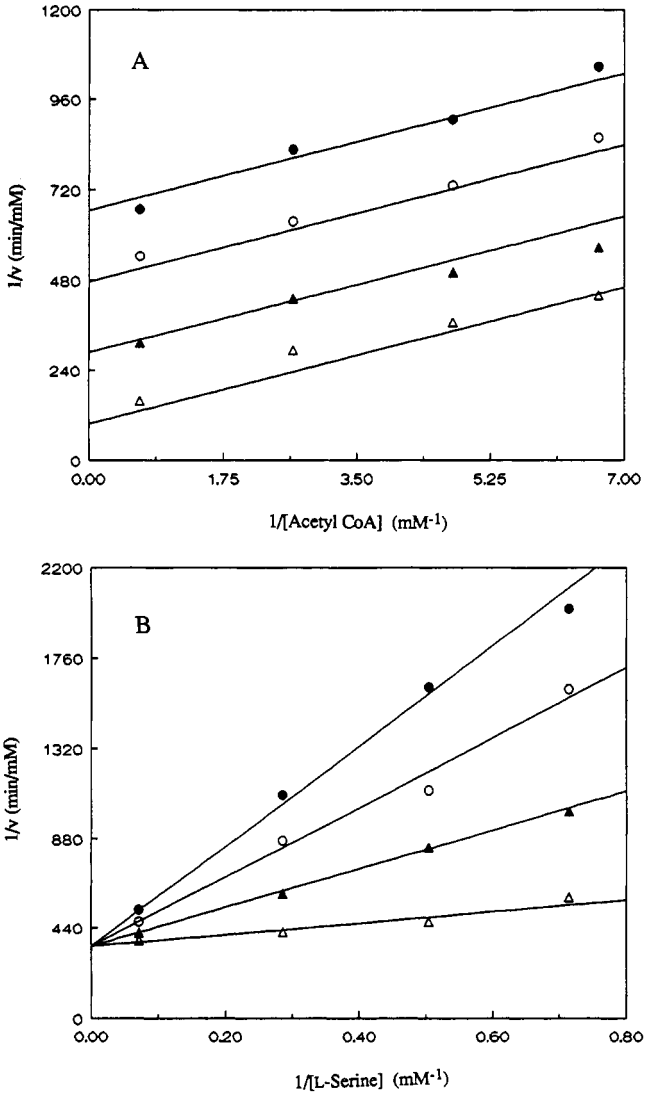


FIGURE 2: Dead-end inhibition by glycine with respect to acetyl CoA (A) or L-serine (B) as the variable substrate. Assays were carried out in 100 mM Hepes, pH 7, at 25 °C. (A) The [glycine] were 60 (●), 40 (○), 20 (▲), and 3 (△) mM; the [acetyl CoA] as indicated and [L-serine] was fixed at 1.5 mM. (B) The [glycine] were 90 (●), 60 (○), 30 (▲), and 3 (△) mM; the [L-serine] as indicated and [acetyl CoA] was fixed at 0.3 mM. Points are experimental and the solid lines are from a fit using eqs 3 and 2, respectively. A STA concentration of 29 ng was used for each assay.

The Michaelis constants obtained for acetyl CoA and L-serine are comparable to the apparent K_m reported by Kredich and Tomkins (1966) when the concentrations of the fixed substrate used in the latter are considered. In the latter report, K_m for L-serine was 0.56 mM with acetyl CoA at a concentration of 0.10 mM and the K_m for acetyl CoA was 0.11 mM with L-serine at a concentration of 1.0 mM. The true K_A can be calculated by multiplying apparent K_A with $(1 + K_B/[B])$ and vice versa. The K_{serine} and $K_{acetyl\ CoA}$ thus obtained are 2.8 and 0.27 mM, respectively, compared to values of 1.4 and 0.4 mM reported in Table 2.

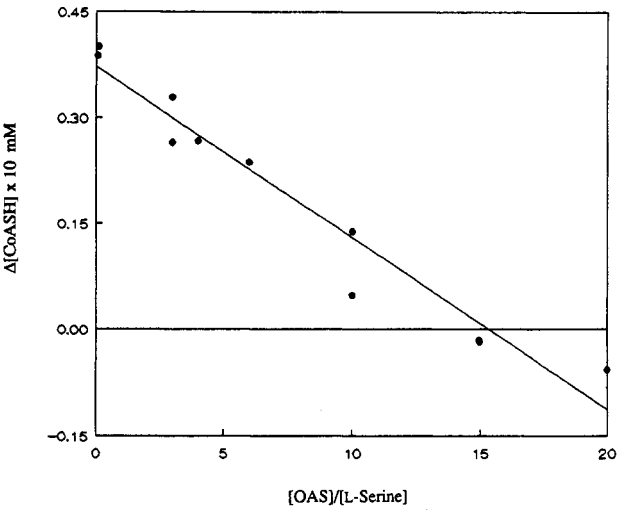
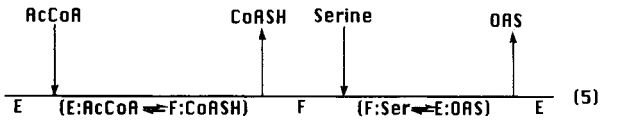


FIGURE 3: The plot of Δ[CoASH] vs [OAS]/[serine] for acquiring the equilibrium constant of the STA reaction. A linear regression of the data gave a [OAS]/[serine] value of 15.4 at a Δ[CoASH] of 0, from which a K_{eq} of 15.4 × ([CoASH]/[acetyl CoA]) or 15.1 was obtained.



Product Inhibition. In a ping pong mechanism, the product of the second half-reaction, in this case OAS, is expected to give competitive inhibition vs acetyl CoA since both bind to E and noncompetitive inhibition vs serine since they bind to different enzyme forms, E and F, and OAS can reverse the second half-reaction producing more F. The expected product inhibition patterns are observed.

In the direction of OAS deacetylation, the results are also consistent with a ping pong mechanism in which OAS is the substrate with the acetyl group to be transferred and CoASH is the acceptor. The competitive nature of the inhibition by L-serine occurs because each acetyl enzyme intermediate that is formed can react either with CoASH to give product or with L-serine to give reactant. The noncompetitive nature of inhibition with respect to OAS by L-serine results from the backward reaction of the acetyl enzyme with L-serine which

will consume a fraction of the acetyl enzyme that is formed, an effect that cannot be overcome by increasing the OAS concentration.

Dead-End Inhibition. Glycine is competitive with L-serine, indicating that both bind to F. In agreement with the latter glycine is uncompetitive vs acetyl CoA which binds to E. Inhibition experiments conducted with *S*-methyl-L-cysteine indicate that it is competitive with respect to OAS and uncompetitive with respect to CoASH in the reverse reaction. The latter data are consistent with the ping pong mechanism with *S*-methyl-L-cysteine combining to the same form of enzyme as does OAS, that is free enzyme. Moreover, two estimates of the true K_i value can be obtained from the dead-end inhibition patterns. The competitive pattern give the true K_{is}, while the uncompetitive pattern also provides an estimate once the K_i value is corrected for the presence of fixed substrate. The expression relating to the two constants is $K_{ii} = K_i(1 + [S]/K_m)$. The K_{is} obtained for glycine vs acetyl CoA is 8.3 mM, and the K_{ii} obtained for glycine vs L-serine is 7.0 mM when corrected for the presence of acetyl CoA. In the case of SMC, the K_{is} obtained for SMC vs CoASH is 9.6 mM, and K_{ii} obtained for SMC is 8.7 mM when corrected for the presence of OAS. Thus, values of 8.3 and 7.0 mM for glycine and 9.6 and 8.7 mM for SMC are in good agreement when standard errors are taken into consideration.

Haldane Relations. To further verify the internal consistency of the kinetic parameters listed in Table 2, the parameters were used to calculate the equilibrium constant for the overall reaction using the Haldane relationship given in eq 6. Considering the fact that the reaction rate is about 65% as fast in phosphate buffer used for the reverse reaction as it is in Hepes buffer used for the forward reaction, the agreement of the calculated K_{eq} with the equilibrium constant obtained by direct measurement is satisfactory and consistent with the proposed mechanism.

$$K_{eq} = [\text{OAS}][\text{CoASH}]/[\text{acetyl CoA}][\text{L-serine}]$$

$$= (V_f)^2 K_{\text{OAS}} K_{\text{CoASH}}/(V_r)^2 K_{\text{acetyl CoA}} K_{\text{L-serine}} = 40 \pm 2 \quad (6)$$

Kinetic Mechanisms of Similar Enzymes. Very little is known of the mechanisms of acetyltransferases in which an acyl group is transferred from acetyl CoA to an alcohol. There are really only three enzymes of this class that have been studied to any extent, viz. homoserine acetyltransferase, chloramphenicol acetyltransferase, and choline acetyltransferase. These three enzymes catalyzed the transfer of an acetyl group from acetyl CoA to, respectively, the γ-hydroxyl of L-homoserine to give *O*-acetyl-L-homoserine (Rowbury, 1961), the 3-hydroxyl of the antibiotic chloramphenicol to give 3-acetylchloramphenicol which is biologically inactive (Shaw, 1983), and the 2-hydroxyl of choline to give acetylcholine. The homoserine acetyltransferases from *Brevibacterium flavum* (Miyajima & Shiio, 1973) and *Bacillus polymyxa* (Wyman & Paulus, 1975) have been suggested to have a ping pong kinetic mechanism in which acetyl CoA acetylates enzyme and CoASH is released prior to homoserine binding. Michaelis-Menten kinetics are observed with each of the two substrates. A similar kinetic mechanism has been proposed for the homoserine acetyltransferase from *Neurospora crassa* (Nagai & Flavin, 1967) and *Bacillus subtilis* (Brush & Paulus, 1971), and for homoserine transsuccinylases from *S. typhimurium* (Brush & Paulus, 1971) and *Escherichia coli* (Lee et al., 1966).

The chloramphenicol acetyltransferases have been studied to an even lesser extent. The enzyme from *Escherichia coli* was found to have a kinetic mechanism which most closely approximated rapid equilibrium random (Kleanthous & Shaw, 1984). This observation, however, does not rule out an acetyl enzyme intermediate such as that predicated in the case of the homoserine acetyltransferases.

Kinetic studies of choline acetyltransferase have been performed on the enzyme from various sources. Although four different kinetic mechanisms have been postulated, a random kinetic mechanism in which the pathway with acetyl CoA binding before choline predominates and approximates a Theorell-Chance mechanism best describes the catalytic behavior of choline acetyltransferase (Hersh, 1982).

The kinetic experiments for STA described above are indicative of a ping pong mechanism in which an acetylenzyme intermediate is produced. The mechanism is thus similar to that of homoserine transacetylase.

ADDED IN PROOF

Recent preliminary studies have shown that STA catalyzes the hydrolysis of acetyl CoA. Monitoring the time course for hydrolysis by coupling the production of CoASH to the production of TNB using DTNB gives a biphasic time course with an initial burst followed by a slow steady-state rate. No significant TNB formation is observed in the absence of acetyl CoA, which monitors the modification of enzyme thiols, or in the absence of STA, which determines the amount of CoASH in the acetyl CoA stock solution. The burst phase likely indicates the initial formation of the acetyl enzyme, followed by a slower rate of hydrolysis of the acetyl enzyme. In this regard, the stoichiometry of TNB formed in the burst phase to enzyme subunits is close to 1. Observation of the above reaction allows a study of the first half-reaction by a combination of steady-state and pre-steady-state kinetics and these studies are planned. In addition, the hydrolysis of the putative acetyl enzyme appears to be slow enough that the intermediate may be isolable, particularly at low temperatures. The lack of observation of TNB produced when STA alone is used in the presence of DTNB indicates that the acetyl enzyme intermediate is either not a thioester or that the active site thiol is not readily modified by the large negatively charged DTNB.

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