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Inactivation of the Amidotransferase Activity of Carbamoyl Phosphate Synthetase by the Antibiotic Acivicin*

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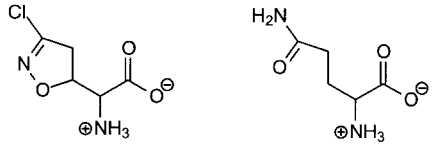
Carbamoyl phosphate synthetase (CPS) from *Escherichia coli* catalyzes the formation of carbamoyl phosphate from 2 mol of ATP, bicarbonate, and glutamine. CPS was inactivated by the glutamine analog, acivicin. In the presence of ATP and bicarbonate the second-order rate constant for the inactivation of the glutamine-dependent activities was 4.0×10^4 M^{-1} s^{-1}. In the absence of ATP and bicarbonate the second-order rate constant for inactivation of CPS was reduced by a factor of 200. The enzyme was protected against inactivation by the inclusion of glutamine in the reaction mixture. The ammonia-dependent activities were unaffected by the incubation of CPS with acivicin. These results are consistent with the covalent labeling of the glutamine-binding site located within the small amidotransferase subunit. The binding of ATP and bicarbonate to the large subunit of CPS must also induce a conformational change within the amidotransferase domain of the small subunit that enhances the nucleophilic character of the thiol group required for glutamine hydrolysis. The acivicin-inhibited enzyme was crystallized, and the three-dimensional structure was determined by x-ray diffraction techniques. The thiol group of Cys-269 was covalently attached to the dihydroisoxazole ring of acivicin with the displacement of a chloride ion.

Acivicin (**I**) is a heterocyclic analog of glutamine (**II**) with anti-cancer and anti-tumor activity that was initially discovered by screening fermentation broths of *Streptomyces sviceus* (1–3). Administration of acivicin stops tumor growth in leukemia, melanoma, and a variety of carcinomas (4, 5), and continued administration of acivicin reduces tumor size and restores muscle mass. Unfortunately, acivicin also causes many undesirable gastrointestinal and neurological side effects. The neurotoxic effects at low concentrations of acivicin include lethargy, depression, insomnia, headaches, confusion, mood swings, vivid dreams, and "large thoughts" (6–8). Higher concentrations of acivicin produce more severe neurological effects such as hallucinations, ataxia, paranoia, amnesia, hostility, and transient unconsciousness (9, 10). These neurological side effects take 24–72 h to manifest themselves and do not correlate with peak levels of acivicin, suggesting that the drug is metabolized into a neurotransmitter antagonist (4).



STRUCTURE 1

Rapidly proliferating tumor cells have elevated activities of the glutamine amidotransferases involved in purine and pyrimidine biosynthesis. Consequently, these cells consume significant amounts of glutamine and deplete this amino acid from the blood (4, 5, 11, 12). Acivicin is a glutamine antagonist that irreversibly inactivates many of the glutamine amidotransferases including formylglycinamidine ribonucleotide synthetase (13), 5-phosphoribosyl 1-pyrophosphate amidotransferase (14), CTP synthetase (15), GMP synthetase (16), and CPS[1] (17–20). All of the glutamine amidotransferases possess an essential cysteine residue that initiates an attack on the amide carbonyl of glutamine to form a covalent γ-glutamyl thioester intermediate. Acivicin contains a 4,5-dihydroisoxazole ring substituted with a chloride that is readily displaced (21–23). The activated thiolate anion of the essential cysteine residue within the amidotransferase family of enzymes has been shown with some of these enzymes to covalently react with the dihydroisoxazole ring of acivicin, resulting in irreversible inactivation.

The inactivation by acivicin of IGP synthetase (24), GMP synthetase (25), CPS II (26), CPS III (27), anthranilate synthetase, and glutamate synthase (28) has been described. Acivicin stoichiometrically inactivates the glutamine-dependent activities of IGP synthetase, GMP synthetase, CPS III, and anthranilate synthetase by alkylation of the nucleophilic cysteine residue located within the active site of these enzymes (24, 25, 27, 28). The ammonia-dependent activities are unaffected by the covalent modification with acivicin, and the rate of inactivation is facilitated by the presence of co-substrates. For example, the addition of ATP and xanthosine monophosphate greatly accelerates the rate of inactivation of GMP synthetase by acivicin (25).

CPS of *Escherichia coli* is a heterodimeric enzyme that catalyzes the formation of carbamoyl phosphate from bicarbonate,

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The atomic coordinates and structure factors (code 1KEE) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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[1] The abbreviations used are: CPS, carbamoyl phosphate synthetase; IGP, imidazole glycerol phosphate.

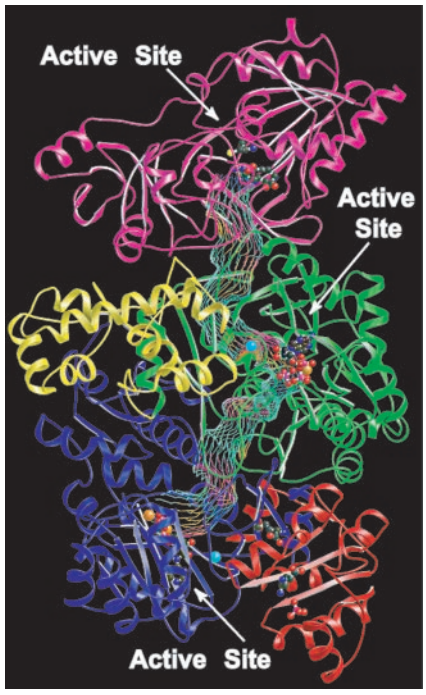


FIG. 1. **Ribbon representation of an α,β-heterodimer of CPS.** The small subunit is displayed in *magenta*. The large subunit is color-coded in *green*, *yellow*, *blue*, and *red* to indicate the carboxy phosphate, the oligomerization, the carbamoyl phosphate, and the allosteric subdomains, respectively. The tunnel connecting the three active sites is displayed in a wire mesh representation.

glutamine, and two molecules of ATP. The synthesis of carbamoyl phosphate is accomplished via four separate reactions occurring at three physically distinct active sites connected by a tunnel that is ~100 Å in length and through which intermediates are translocated (29, 30). As indicated in Fig. 1, the tunnel begins at the glutamine-binding site within the small subunit and runs through the interior of the large subunit where it connects with the two binding sites for ATP. Cys-269 of the small subunit has been shown to function as the nucleophile that attacks the amide carbonyl of glutamine to form the γ-glutamyl thioester intermediate. The ammonia is subsequently channeled to the large subunit (31–33). The binding site for glutamine is largely hydrophilic with numerous electrostatic interactions between the bound substrate and the protein matrix.

Acivicin is an extraordinarily potent inactivator of glutamine amidotransferases. To further understand the high affinity and selectivity of acivicin for the active sites of these enzymes, the molecular interactions between the enzyme and inhibitor must be delineated in greater detail. A theoretical model for the binding of acivicin to the amidotransferase domain of GMP synthetase has been published (24). In this paper, a detailed kinetic analysis of acivicin inactivation of CPS and the high resolution x-ray crystal structure of the covalently modified enzyme are presented.

EXPERIMENTAL PROCEDURES

Materials—Carbamoyl phosphate synthetase was isolated from *E. coli* as described previously (34). Acivicin was purchased from Sigma, and all other reagents and coupling enzymes were obtained from commercial sources.

Enzyme Assays—A continuous spectrophotometric assay was used to monitor the production of glutamate following the enzymatic hydrolysis of glutamine. Carbamoyl phosphate synthetase (6–14 nmol) was added to an assay solution composed of 0.1 M Hepes (pH 7.6, 25 °C), 100 mM KCl, 10 units of glutamate dehydrogenase, and 1.0 mM 3-acetylpyrimidine adenine nucleoside and varying amounts of glutamine. The glutamate concentration was calculated using an extinction coefficient for the reduced form of 3-acetylpyrimidine adenine nucleoside of 8.3 mM^{-1} cm^{-1} at 363 nm. The rate of glutamate formation when ATP and bicarbonate were present was determined using the same assay solution but supplemented with the addition of 5.0 mM ATP, 10 mM $MgCl_2$, 10 mM ornithine, and 40 mM bicarbonate.

The rate of ADP formation was assayed using a pyruvate kinase/lactate dehydrogenase coupled assay by following the loss of NADH spectrophotometrically at 340 nm (35). The assay solution consisted of 0.1 M Hepes (pH 7.6, 25 °C), 1.0 mM phosphoenol pyruvate, 0.3 mM NADH, 0.1 M KCl, 20 mM ornithine, 20 mM $MgCl_2$, 10 mM ATP, and 33 μg of pyruvate kinase and lactate dehydrogenase. For the glutamine-dependent utilization of ATP, 10 mM glutamine was added to the assay solutions.

Inactivation of CPS by Acivicin—The rate of inactivation of the glutaminase and glutamine-dependent ATPase activities of CPS by acivicin was determined by incubating the enzyme (20 μM) in 0.1 M Hepes buffer (pH 7.6, 25 °C) with varying amounts of the inhibitor. At various time intervals, the aliquots were removed and diluted at least 100-fold into the appropriate assay solution. The time course for the fraction of the initial catalytic activity remaining (f) was a pseudo-first-order decay, and the rate constant k was obtained by a fit of the data to Equation 1. The plots of the pseudo-first-order rate constants *versus* the initial acivicin concentration yielded second-order rate constants for the inactivation of CPS.

$$f = e^{-kt} \quad \text{(Eq. 1)}$$

Effect of ATP and Bicarbonate on the Rate of Acivicin Modification of CPS—The rate of inactivation of the glutamine-dependent ATPase activity of CPS by acivicin and the rate of activation of the bicarbonate-dependent ATPase activity of CPS in the presence of 10 mM ATP and 40 mM bicarbonate were investigated. For the inactivation of the glutamine-dependent ATPase activity, the time courses for the fraction activity remaining were pseudo-first-order and were analyzed as described previously. The time course for acivicin activation of the bicarbonate-dependent ATPase activity was also pseudo-first-order, and the rate constant k was obtained by a fit of the data to Equation 2. In this equation v is the observed velocity, A is the amplitude factor, k is the rate constant of activation, and v_o is the velocity observed when no acivicin is added. A plot of the pseudo-first-order rate constants as a function of the acivicin concentration yielded the second-order rate constant for activation.

$$v = A(1 - e^{-kt}) + v_o \quad \text{(Eq. 2)}$$

Partition Ratio for Acivicin Inactivation of CPS—The partition ratio for the inactivation of CPS with acivicin was determined. In these experiments CPS (20 μM) was incubated at 25 °C with various concentrations of acivicin in 0.1 M Hepes buffer (pH 7.6). After 6 h of incubation, the aliquots were removed, and the catalytic activity remaining was determined.

Structural Analysis—For crystallization trials, the protein was concentrated to 4 mg/ml in 10 mM Hepes (pH 7.0) and 100 mM KCl and incubated overnight with 20 mM acivicin. Large single crystals were grown at 4 °C using a precipitant solution containing 0.65 M tetraethylammonium chloride, 8% (w/v) poly(ethylene glycol) 8000, 100 mM KCl, 0.5 mM $MnCl_2$, 0.5 mM ornithine, 1.25 mM ADP, and 25 mM Hepes (pH 7.4). The resulting crystals belonged to the space group $P2_12_12_1$ with unit cell dimensions of a = 152.4 Å, b = 164.4 Å, and c = 333.2 Å and one α/β-heterotetramer/asymmetric unit.

An x-ray data set to a nominal resolution of 2.1 Å was collected at the Stanford Synchrotron Radiation Laboratory on beam-line 7-1 with the MAR300 image plate system. Prior to x-ray data collection, the crystals were flash frozen according to previously published procedures (29). All x-ray data frames were processed with DENZO and scaled with SCALEPACK (36). Relevant x-ray data collection statistics are given in Table I. The structure of the complex between CPS and acivicin was solved by difference Fourier techniques. Alternate cycles of least squares refinement with the program TNT (37) and manual model building with the program TURBO (38) reduced the R factor to 18.6% for all measured x-ray data from 30 to 2.1 Å. Least squares refinement statistics are presented in Table II.

RESULTS

Inactivation of CPS by Acivicin—Acivicin rapidly inactivates the glutamine-dependent activities of CPS. The diminution in

TABLE I
X-ray data collection statistics

Resolution	Independent reflections	Completeness	Redundancy	Average *I*/average σ(I)	R_{sym}[a]
Å		%			%
30.0–2.10	429,957	88.9	4.4	37.9	4.4
2.18–2.10[b]	34,861	72.7	2.5	5.9	17.9

[a] $R_{sym} = (\Sigma|I - \bar{I}|/\Sigma I) \times 100$.
[b] Statistics for the highest resolution bin.

TABLE II
Relevant least-squares refinement statistics

Resolution limits (Å)	30.0–2.10
R factor (overall) %/no. reflections[a]	18.6/429957
R factor (working) %/no. reflections	18.7/386960
R factor (free) %/no. reflections	21.1/42997
No. protein atoms	44346
No. hetero-atoms	4550[b]
Weighted root mean square deviations from ideality	
Bond lengths (Å)	0.010
Bond angles (deg)	2.12
Trigonal planes (Å)	0.006
General planes (Å)	0.010
Torsional angles (deg)	17.7

[a] *R* factor = $(\Sigma|F_o - F_c|/\Sigma|F_o|) \times 100$ where F_o is the observed structure-factor amplitude and F_c is the calculated structure-factor amplitude.
[b] These include 4176 water molecules, 8 ADP molecules, 4 tetraethylammonium ions, 22 chloride ions, 12 manganese ions, 32 potassium ions, 4 phosphates, and 4 ornithine molecules.

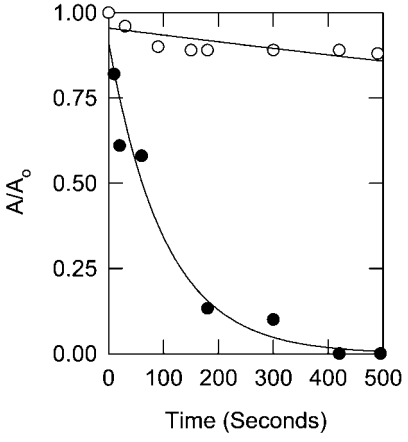


FIG. 2. **Inactivation of glutaminase activity of CPS by the addition of acivicin.** ●, CPS was incubated with 5.0 mM acivicin, and the relative rate of glutamine hydrolysis was measured as a function of time. The data were fit to Equation 1 with a rate constant of 0.8 min^{-1}. ○, protection against inactivation by acivicin through the addition of 1.0 mM glutamine to the incubation mixture. The data were fit to Equation 1 with a rate constant of 0.012 min^{-1}.

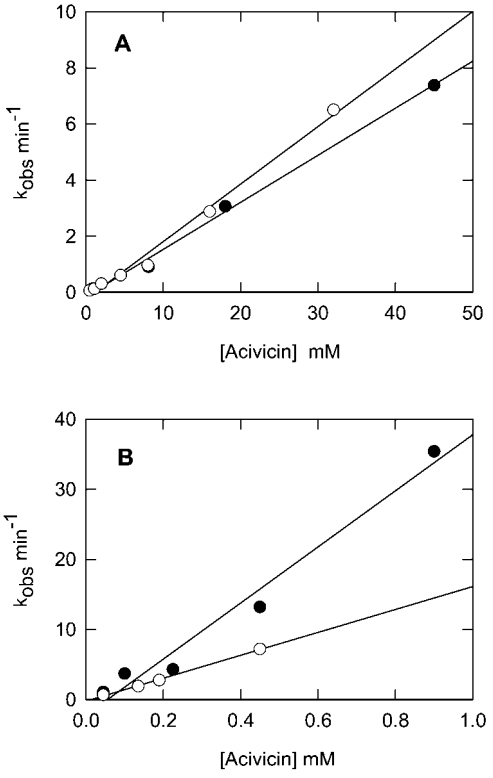


FIG. 3. **Determination of the second-order rate constants for the modification of CPS by acivicin.** *A*, the first-order rate constants for the inactivation of CPS by acivicin are plotted as a function of the initial acivicin concentration. ●, the second-order rate constant for inactivation of the glutaminase activity was determined to be 168 M^{-1} min^{-1}. ○, the second-order rate constant for inactivation of the glutamine-dependent turnover of ATP was 200 M^{-1} min^{-1}. *B*, the first-order rate constants for the inactivation of CPS by acivicin in the presence of 10 mM ATP and 40 mM bicarbonate. ●, the second-order rate constant for inactivation of the glutaminase activity was 40,000 M^{-1} min^{-1}. ○, the second-order rate constant for the activation of the bicarbonate-dependent ATPase activity was 16,000 M^{-1} min^{-1}.

the ability of CPS to hydrolyze glutamine after the addition of acivicin is presented in Fig. 2. At a concentration of 5.0 mM acivicin, the loss in catalytic activity was first-order, and the data were fitted to Equation 1 with a rate constant of 0.8 min^{-1}. The time courses for the inactivation of the glutamine-dependent ATPase activity of CPS by acivicin were also first-order (data not shown). Glutamine was shown to protect the enzyme from inactivation, thus demonstrating that the inactivation event was due to modification of an active site residue within the binding site for glutamine (Fig. 2). The first-order rate constants for inactivation of the glutaminase and glutamine-dependent hydrolysis of ATP were plotted as a function of the initial acivicin concentration (Fig. 3*A*). In both of these plots there was no evidence to indicate saturation of the enzyme by acivicin at concentrations up to 45 mM. The apparent second-order rate constant for the inactivation of the glutamine-dependent ATPase activity of CPS was found to be 204 ± 10 M^{-1} min^{-1}. The second-order rate constant for inactivation of the glutaminase activity of CPS by acivicin was 168 ± 6 M^{-1} min^{-1}. The rate of inactivation of CPS by acivicin was greatly enhanced in the presence of added ATP and bicarbonate (Fig. 3*B*). The rate of inactivation of the glutamine-dependent ATPase activity was $4.0 \pm 0.4 \times 10^4$ M^{-1} min^{-1} in the presence of 10 mM ATP and 40 mM HCO_3^-. This is a ~200-fold enhancement over the second-order rate constant determined in the absence of these substrates. There was no indication of saturation by acivicin in this plot.

The acivicin-treated enzyme had an increase in the rate of the bicarbonate-dependent ATPase activity. The effect of acivicin on the bicarbonate-dependent ATPase activity of CPS as a function of time is shown in Fig. 4. The rate of activation of the bicarbonate-dependent ATPase activity in the presence of 10 mm ATP and 40 mM bicarbonate at various acivicin concentrations was determined, and the apparent second-order rate con-

stant was equal to $1.6 \pm 0.1 \times 10^4$ M^{-1} min^{-1} (Fig. 3*B*).

Partition Ratio for Acivicin Inactivation of CPS—The efficiency for the inactivation of CPS by acivicin was determined by incubating a fixed concentration of enzyme with stoichiometric amounts of the inactivator. A plot of the fractional activity remaining after 6 h of incubation is presented in Fig. 5. Extrapolation of the data collected at substoichiometric amounts of acivicin indicated that ~1.7 molecules of acivicin were required to inactivate one molecule of CPS.

Steady State Parameters for the Acivicin-treated Enzyme—Carbamoyl phosphate synthetase was inactivated by acivicin, and the kinetic constants (K_m and k_{cat}) for the modified enzyme were determined for the bicarbonate-, glutamine-, and ammonia-dependent ATPase activities of CPS. The results are presented in Table III.

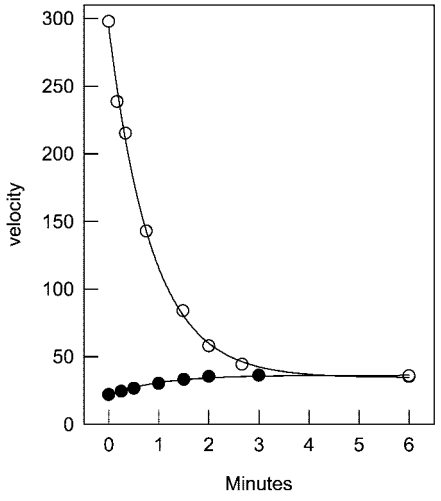


FIG. 4. **Effect of acivicin on the bicarbonate-dependent ATPase reaction and the glutamine-dependent ATPase reaction of CPS.** CPS was incubated with 45 mM acivicin, 10 mM ATP, and 40 mM bicarbonate. The aliquots were removed at various times, and the activity was determined for the bicarbonate-dependent ATPase reaction (●) and the glutamine-dependent ATPase reaction (○).

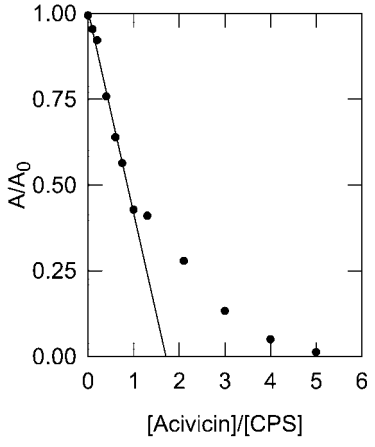


FIG. 5. **Determination of the partition ratio for inactivation of CPS by acivicin.** The fraction of catalytic activity remaining as a function of the ratio of initial concentration of acivicin to CPS is shown. The partition ratio was 1.7.

DISCUSSION

Acivicin is a glutamine analog that irreversibly inactivates glutamine amidotransferases. It has been shown to stoichiometrically react with the active site cysteine residues in IGP synthetase (24), GMP synthetase (25), and anthranilate synthetase (28) and to destroy the ability of these enzymes to utilize glutamine as an ammonia source. However, the ability of these modified enzymes to utilize free ammonia is relatively unchanged. In this study, acivicin was also shown to irreversibly inactivate the glutamine-dependent activities of CPS from *E. coli* but with the ammonia-dependent activities left unaffected. As with other amidotransferases, glutamine protected the enzyme from inactivation (Fig. 2).

Previous studies have demonstrated that the hydrolysis of glutamine by CPS involves the formation of a γ-glutamyl thioester intermediate (39, 40). In the absence of ATP and bicarbonate, the second-order rate constant for the formation of the γ-glutamyl thioester intermediate is 3,400 M^{-1} min^{-1}. The rate of hydrolysis of this intermediate is 0.17 min^{-1}, which is rate-determining (35). The additions of ATP and bicarbonate increase the rate of glutamine hydrolysis 700-fold with a change in the rate determining step toward the rate of formation of the thioester intermediate (35). Pseudo-first-order kinetics were observed for the inhibition of CPS by acivicin. The apparent second-order rate constant for acivicin inactivation of the glutamine-dependent activities of CPS in the absence of ATP and bicarbonate is ~200 M^{-1} min^{-1}. When ATP and bicarbonate are added, the apparent second-order rate constant is increased to $\sim 4.0 \times 10^4$ M^{-1} min^{-1}. The binding of ATP and subsequent reaction with bicarbonate within the large subunit of CPS must therefore induce conformational changes in the glutamine amidotransferase site. These alterations in protein structure promote a 700-fold increase in the rate of glutamine hydrolysis and a 200-fold increase in the rate of covalent modification by acivicin. Acivicin did not exhibit saturation kinetics, suggesting that this inhibitor is initially rather loosely bound within the active site (Fig. 3). Similarly, for inactivation of GMP synthetase by acivicin, saturation kinetics were not observed (25). In contrast, acivicin inactivation of IGP synthetase did exhibit saturation kinetics (24). A comparison of the inactivation rate constants for acivicin with CPS, IGP synthetase, and GMP synthetase is presented in Table IV.

Chemical reagents that modify Cys-269 of CPS inactivate all of the glutamine-dependent activities and induce a modest increase in the rate of the bicarbonate-dependent ATPase activity (31, 32, 41–44). Acivicin modification of CPS produces a 3-fold increase in the value of k_{cat}/K_m for the bicarbonate-dependent ATPase activity. These results indicate that occupancy of the glutamine-binding site can enhance the reactivity of the carboxy phosphate intermediate bound within the large sub-

TABLE III
Kinetic parameters for acivicin-treated CPS
The rate of formation of ADP was monitored with variable concentrations of ATP at pH 7.6, 25 °C.

	NH_3^--dependent ATPase	Glutamine-dependent ATPase	HCO_3^--dependent ATPase
Wild type CPS			
K_m (μM)	60 ± 11	201 ± 24	24 ± 2
k_{cat} (min^{-1})	174 ± 6	336 ± 5	13 ± 1
Acivicin-treated CPS			
K_m (μM)	77 ± 5	16 ± 2	11 ± 2
k_{cat} (min^{-1})	146 ± 2	18 ± 1	18 ± 1

TABLE IV
Comparison of the kinetic parameters inactivation of IGP synthase, GMP synthase, and CPS

	Substrates	Acivicin inactivation	Glutaminase activity (k_{cat}/K_m)
S. cerevisiae CPS[a]	− ATP, HCO_3^-	2.0×10^2 M^{-1} min^{-1}	1.0×10^3 M^{-1} min^{-1}
	+ ATP, HCO_3^-	4.0×10^4 M^{-1} min^{-1}	2.0×10^6 M^{-1} min^{-1}
E. coli IGP synthetase[b]	− PRFAR[c]	1.6×10^2 M^{-1} min^{-1}	9.0×10^2 M^{-1} min^{-1}
	+ PRFAR	6.0×10^5 M^{-1} min^{-1}	2.3×10^6 M^{-1} min^{-1}
S. cerevisiae IGP synthetase[b]	− PRFAR	9.0×10^1 M^{-1} min^{-1}	1.0×10^2 M^{-1} min^{-1}
	+ PRFAR	1.7×10^4 M^{-1} min^{-1}	1.9×10^5 M^{-1} min^{-1}
GMP synthetase[b]	− ATP, XMP		Undetectable
	+ ATP, XMP	1.1×10^6 M^{-1} min^{-1}	1.3×10^6 M^{-1} min^{-1}

[a] Miles *et al.* (35).
[b] Chittur *et al.* (24).
[c] PRFAR, *N*′-(5′-phosphoribulosyl-formimino-5-aminoimidazole-4-carboxamide ribonucleotide.
[d] XMP, xanthosine monophosphate.

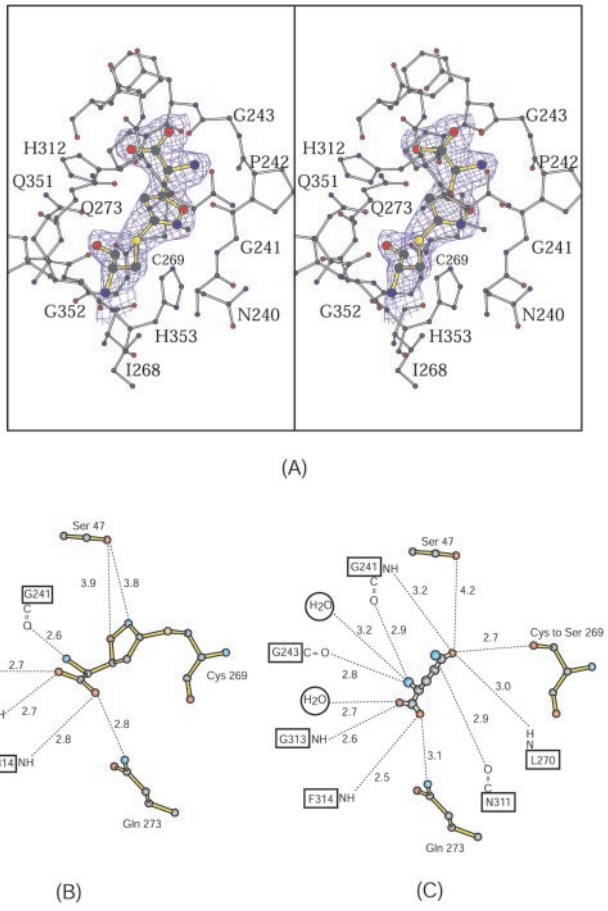


FIG. 6. **The binding of acivicin within the CPS small subunit.** Electron density corresponding to the bound inhibitor is shown in *A*. The electron density map was contoured at 3 σ and calculated with coefficients of the form ($F_o - F_c$), where F_o was the native structure factor amplitude and F_c was the calculated structure factor amplitude. Coordinates for the acivicin moiety and Cys-269 were not included in the Fourier synthesis. A schematic of the hydrogen bonding interactions between the inhibitor and the protein is given in *B* for comparison with that previously found for the binding of glutamine to the C269S mutant of CPS in *C*.

unit of CPS. This rate enhancement must be promoted through an induced conformational change.

A stereo view of the electron density corresponding to the bound inhibitor in the small subunit of CPS is given in Fig. 6*A*. As can be seen, the electron density is well ordered and indicates, as expected, that the dihydroisoxazole ring is covalently

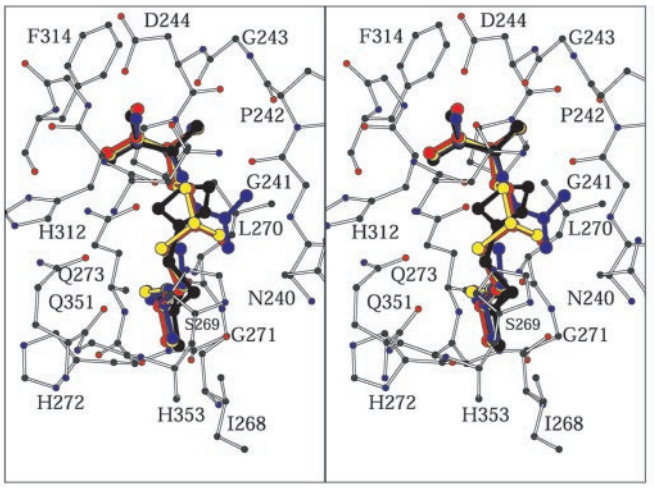
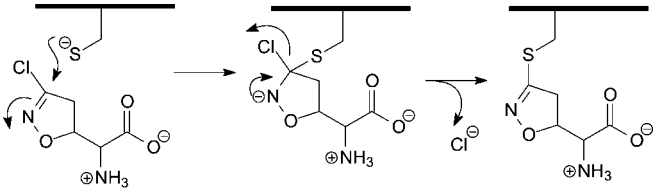


FIG. 7. **Comparison of the ligand-binding modes in the CPS small subunit.** The observed conformations of glutamate, glutamate γ-semialdehyde, glutamyl thioester, and acivicin, when bound in the active site of the small subunit, are indicated in *blue*, *yellow*, *red*, and *black*, respectively.



SCHEME 1

attached to the thiolate of Cys-269. A cartoon of the hydrogen bonding pattern formed between the inhibitor and the protein is presented in Fig. 6*B*. The α-carboxylate group of acivicin is anchored to the protein via the backbone NH peptidic groups of Gly-313 and Phe-314. A bound water molecule and $N^{\epsilon 2}$ of Gln-273 provide additional hydrogen bonds to the carboxylate moiety of the inhibitor. The α-amino group of the ligand is positioned within 2.6 Å of the carbonyl group of Gly-241. Ser-47 lies at ~3.9 Å from the oxygen and nitrogen atoms of the dihydroisoxazole ring. It has been postulated that during the hydrolysis of glutamine to glutamate in CPS, O^{γ} of Ser-47 serves both to position the carbonyl carbon of the glutamine side chain for nucleophilic attack by the thiolate of Cys-269 and to stabilize the developing oxyanion (30). For comparison purposes, a cartoon of the hydrogen bonding pattern observed between CPS (C269S mutant) and glutamine is also presented in Fig. 6*C*. As can be seen, there are eight direct interactions within 3.2 Å between the protein and glutamine in contrast to the four observed in the acivicin-bound enzyme. The replacement of the carboxamide group of glutamine with the dihydroisoxazole ring in acivicin results in the loss of three key interactions with two backbone peptidic NH groups (Gly-241 and Leu-270) and the carbonyl oxygen of Asn-311. Additionally, the hydrogen bond between the α-amino group of glutamine and the carbonyl oxygen of Gly-243 is lost upon complexation with acivicin. The less extensive hydrogen bonding network between the protein and acivicin may, in part, explain the lower affinity of the protein for the inhibitor. Other than these

[2] Since the submission of this manuscript, Davisson and co-workers (46) have reported the crystal structure of imidazole glycerol phosphate synthase with acivicin covalently attached to the active site cysteine in the glutamine binding site. This structure nicely illustrates the use of the interior of a TIM barrel domain as an integral part of a molecular tunnel for the delivery of ammonia.

changes, however, the polypeptide chains for the small subunits complexed with either acivicin or glutamine are remarkably similar and superimpose with a root mean square deviation of 0.23 Å for all atoms.[2]

Previous biochemical studies of CPS have suggested that the hydrolysis of γ-glutamine to glutamate and ammonia proceeds through two tetrahedral adducts and a glutamyl-thioester intermediate. By the judicious use of substrate analogs and site-directed mutant proteins, it has been possible to define, by high resolution x-ray analyses, the structures of these proposed intermediates (30, 45). To examine the geometry of the Michaelis complex, Cys-269 was replaced with a serine residue and crystallized. To mimic the tetrahedral intermediate, a complex between native CPS and glutamate γ-semialdehyde was prepared. Finally, to address the geometry of the small subunit active site during the formation of the γ-glutamyl thioester intermediate, His-353 was changed to an asparagine residue. A superposition of these three structures onto the model of the acivicin-inactivated CPS present here is given in Fig. 7. Acivicin adopts a similar conformation in the active site to that observed for both the tetrahedral and γ-glutamyl thioester intermediates. The α-carboxylate and α-amino groups of all of the ligands occupy nearly identical positions. The potency of acivicin as an inhibitor is, indeed, not surprising in light of these structural similarities. A mechanism for the inactivation of CPS by acivicin that is consistent with the x-ray structure of the inactivated enzyme is presented in Scheme 1.

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ENZYME CATALYSIS AND REGULATION:

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