Escherichia coli thymidylate synthase: Amino acid substitutions by suppression of amber nonsense mutations

(suppressor tRNAs/site-directed mutagenesis/structure/function)

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ABSTRACT   By using site-directed oligonucleotide muta-
genesis, amber nonsense stop codons (5'-TAG-3') have been
introduced at 20 sites in the *Escherichia coli* thymidylate
synthase gene. By transforming the *thyA* mutant plasmids into
13 strains, each of which harbor different amber suppressor
tRNAs, we were able to generate over 245 amino acid substi-
tutions in *E. coli* thymidylate synthase (EC 2.1.1.45). Growth
characteristics of these mutants have been studied, yielding a
body of information that includes some surprising results in
light of the recently published crystal structure of the enzyme.

Escherichia coli thymidylate synthase (EC 2.1.1.45) is re-
quired by the sole *de novo* pathway for the biosynthesis of
thymidine monophosphate (dTMP) from deoxyuridine mono-
phosphate (dUMP) (1). The reaction requires the cofactor
5,10-methylenetetrahydrofolate, which serves both as a re-
ductant and as a one-carbon donor in the methylation reac-
tion. Insights into the enzyme's mechanism of action have
come from the study of model compounds, substrate analogs,
and mechanism-based inhibitors. Because rapidly dividing
cells require an abundant pool of dTMP for the synthesis of
DNA, thymidylate synthase is a choice target enzyme in the
design of chemotherapeutics. For example, the antitumor
agent 5-fluoro-2'-deoxyuridine monophosphate (FdUMP)
mimics dUMP in the catalytic reaction until it is trapped in a
form resembling a steady-state intermediate (2–4). Matthews
et al. (5, 6) have recently determined the structure of *E. coli*
thymidylate synthase complexed with FdUMP and 10-
propargyl-5,8-dideazafolate (a folate analog that inhibits
thymidylate synthase) (7). This has aided our understanding
of how these inhibitors interact with their respective binding
sites and has yielded valuable information on structure–
function relationships among thymidylate synthases in gen-
eral. Their results confirm that Cys-146 is the active-site
nucleophile that initiates the catalytic reaction, and they
identify other amino acids that form substrate binding pock-
ets or comprise important structural elements.

In this investigation we have introduced amber (5'-TAG-3')
nonsense codons at 20 different sites in the *E. coli* thymidy-
late synthase gene and have made 12 or 13 amino acid
substitutions at each site by growing the mutant plasmids in
suppressor strains. These strains carry altered tRNA genes
that have anticodons that base pair with the UAG codons,
allowing the insertion of 1 of 13 different amino acids at each
amber nonsense site. The application of this technique to the
study of the *lac* repressor of *E. coli* has led to the generation
of over 1600 amino acid substitutions in that protein (8, 9).
Here we apply this technique to make amino acid substitu-
tions in *E. coli* thymidylate synthase to produce over 245
variants of this enzyme. Growth characteristics of these
mutants and enzyme assays on a selected set of altered

thymidylate synthases have facilitated a more detailed eval-
uation of the importance of the substituted residues.

MATERIALS AND METHODS

Bacterial Strains and Media. *E. coli* strain XAC is F⁻
Δ(*lacproB*)$_{XIII}$, *nalA*, *rif*, *argE*$_{am}$, *ara*. XAC *thyA* was made
by mutagenizing XAC with the frameshift mutagen ICR 191
and selecting for Thy⁻ colonies on trimethoprim (10). A *recA*
deletion was introduced to create XAC *thyA*, *recA*::Tn*10*.
The chromosomal suppressor strains Su2-89, Su3, Su5RF,
and Su6 have been described (11–13), and *thyA*, *recA*::Tn*10*
constructs were made as described above. Strain CJ236 is
dut-1, *ung-1*, *thi-1*, *relA-1*/pCJ105 (Camʳ), and strain MV1190
is Δ(*lacproAB*), *thi*, *supE*, (*srl-recA*)*306*::Tn*10* (tetʳ) [F':
traD36, *proAB*, *lacIqZ* ΔM15]; both were obtained from
Bio-Rad. All media were as described (10) and were supple-
mented as necessary with proline (100 μg/ml), methionine (50
μg/ml), ampicillin (100 μg/ml), nalidixic acid (30 μg/ml),
chloramphenicol (30 μg/ml), tetracycline (15 μg/ml), or
thymidine (100 μg/ml).

Bacteriophage and Plasmids. Construction of the vector
pGFIB-1 (14) and the plasmid-based suppressors (pGFIB-
Ala, etc.) has been published (15–19). The wild-type *thyA*
gene cloned into pACYC 184 (20) is designated pATAH (21)
and was a gift of M. Belfort (State of New York Department
of Health, Albany, NY). The 1.16-kilobase *Hind*III fragment
containing the *thyA* gene was cloned into M13mp19 to yield
the phage MLM192.

Site-Directed Mutagenesis. Site-directed mutagenesis of
MLM192 was carried out as described (22–24). Mutants were
verified by sequencing (25) and subcloned back into the
*Hind*III site of pACYC 184. Mutants were designated
(pECTS-C146am, etc.). Restriction mapping verified that all
mutants were oriented identically.

Growth Tests. The *thyA* amber mutant plasmids were
transformed into the suppressor strains, and freshly purified
colonies were streaked onto two sets of minimal M9 glucose
plates (10). One set contained all the appropriate supplements
to maintain the strain and plasmids, and the other set con-
tained all the supplements except thymidine.

Extract Preparation. Thirty-milliliter cultures of the bac-
terial strains described above were harvested at an OD$_{600}$ of
≈1. The extract was prepared by resuspending the cell pellet
in 2 ml of 50 mM Tris, pH 7.5/20 mM 2-mercaptoethanol/1
mM phenylmethylsulfonyl fluoride and was kept on ice
throughout the remaining steps. Cells were lysed by sonica-
tion, and the cell debris was removed by centrifugation.
Glycerol was added to the supernatant to a final concentra-
tion of 5% (vol/vol). The protein content of the extracts was
measured with the Bio-Rad protein assay kit.

Measurement of Thymidylate Synthase Activity. Thymidy-
late synthase enzyme activity in the crude extract was mea-
sured by a tritium-release assay described by Roberts (26).

Abbreviation: FdUMP, 5-fluoro-2'-deoxyuridine monophosphate.

For the total activity values of the crude extract shown in Table 3, 1 unit is defined as the release of 1 nmol of tritium per mg of total extract protein in 1 hr at 25°C with 5 μM [5-^{3}H]dUMP. Extracts prepared from *E. coli* containing the wild-type *thyA* gene on the pACYC 184 vector were used as the wild-type standard.

Quantitative Western Analysis of Crude Extracts. The concentration of thymidylate synthase enzyme in the crude extract was determined by quantitative Western blots. Proteins were separated by SDS/polyacrylamide gel electrophoresis and transferred to nitrocellulose filters using the Semi-Phor semidry blotting apparatus (Hoefer). Filters were incubated with anti-thymidylate synthase antisera (kindly provided by Diagnostics Products, Los Angeles) and developed by using goat anti-rabbit ^{125}I-labeled IgG (NEN). Filters were exposed to x-ray film to obtain autoradiographs and quantitated by measuring transmittance on a Bio-Rad model 620 video densitometer.

To standardize densitometry transmittance values with respect to the amount of thymidylate synthase protein, various concentrations of purified thymidylate synthase (kindly provided by Kate Welsh, Agouron Pharmaceuticals) were included in every Western blot.

RESULTS

Site-Directed Mutagenesis. Fig. 1 depicts the method used to introduce amber chain-termination codons (5'-TAG-3') into the *thyA* gene of *E. coli*. Since the efficiency of the
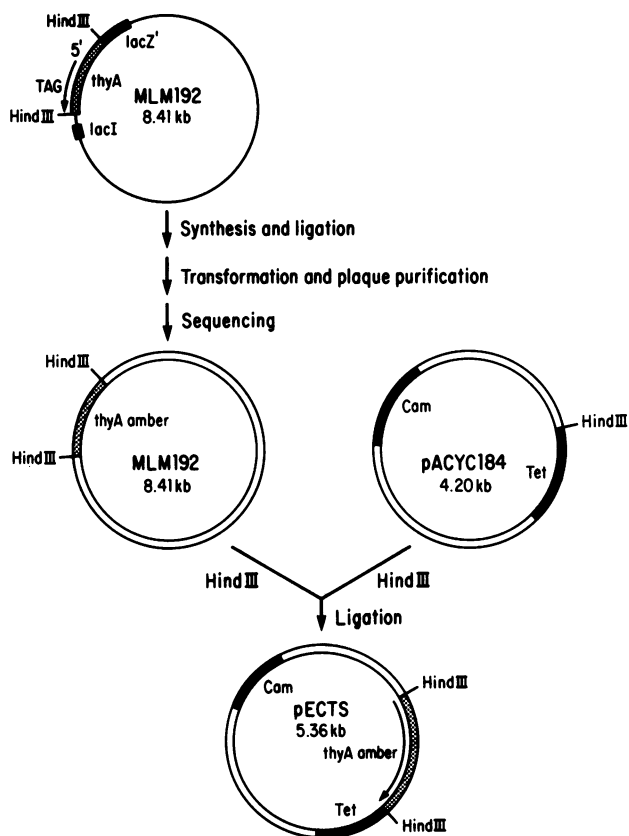

FIG. 1. Construction of pECTS amber mutants. The *thyA*-containing *Hind*III fragment from plasmid pATAH was subcloned into M13mp19 to create MLM192. Oligonucleotides containing amber stop codons (5'-TAG-3') were annealed to the template and extended and ligated with T4 DNA polymerase and ligase. Mutants were identified by sequencing and were subcloned into pACYC 184 to create the pECTS amber mutant series (pECTS-C146am, etc.). kb, Kilobases; Tet, tetracycline-resistance gene; Cam, chloramphenicol-resistance gene.

suppressor is particularly influenced by the sequence of the first two bases following the nonsense codon (11, 27), occasionally additional alterations were made if they did not change the identity of the succeeding amino acid.

Suppression of Amber Mutations. The pECTS amber mutants were transformed into 13 different suppressor strains. Amber nonsense suppressor strains carry alleles of tRNA genes whose anticodon has been altered such that it inserts an amino acid in response to the amber (5'-UAG-3') chain-termination codon. Four suppressors have alterations in chromosomal tRNA genes (Su2-89 *thyA recA*, Su3 *thyA recA*, etc.) (11–13), whereas the rest have been constructed synthetically (15–19) and are on a plasmid vector, pGFIB-1, in strain XAC *thyA recA*. pGFIB-1 suppressor plasmids, which carry the ColE1 origin, form a compatible plasmid system with the pECTS amber plasmids, which carry the origin of replication from plasmid p15A.

Transformation of the 20 amber mutants in *thyA* into the 13 suppressor strains results in over 245 variants of *E. coli* thymidylate synthase. Transformants were isolated and purified and then streaked onto two sets of minimal glucose plates containing supplements necessary to maintain the strains and plasmids but either containing or lacking thymidine as described in *Materials and Methods* (Table 1).

Tests of the Suppressor System. Seven of the 20 amber mutants in *thyA* could accept any of the 13 amino acid substitutions, suggesting that these amino acids were not critical for the function of thymidylate synthase or that a Thy$^+$ revertant had formed. While all the suppressor strains were *thyA recA*, effectively eliminating recombination, we tested for Thy$^+$ revertants by isolating plasmid DNA and retransforming into XAC *thyA recA*. In no case did transformation result in Thy$^+$ colonies. Transformation of these strains with a suppressor plasmid restored the strain to Thy$^+$, verifying that these were highly substitutable sites. Plasmid DNA was also isolated from six other amber plasmids. Again, transformation of XAC *thyA recA* did not result in Thy$^+$ colonies, and when transformed into the suppressor series, they retained their original suppression patterns.

Table 1. Suppression patterns of *E. coli* thymidylate synthase

Wild-type amino acid	Nonpolar					Polar				Basic			Acidic
	Gly	Ala	Pro	Phe	Leu	Cys	Ser	Tyr	Gln	His	Lys	Arg	Glu
Glu-14	+	+	+	+	+	+	+	+	+	+	+	+	+
Arg-21	±	∓	±	−	−	−	±	−	−	±	−	+	−
Phe-30	−	−	−	+	+	−	−	+	−	−	−	−	−
Gln-33	+	+	+	+	+	+	+	+	+	+	+	+	+
Arg-35	+	+	+	+	+	+	+	+	+	+	+	+	±
Trp-80	∓	∓	−	±	−	−	−	−	−	∓	−	−	∓
Asp-81	+	+	+	+	+	+	+	+	+	+	+	+	+
Asp-105	+	+	+	+	+	+	+	+	+	+	+	+	+
Asp-110	−	−	−	−	−	±	±	±	−	−	−	−	−
Asn-121	+	+	+	+	+	+	+	+	+	+	+	+	+
Arg-126	∓	∓	±	−	−	−	±	+	−	+	+	+	−
Arg-127	+	+	+	+	+	+	+	+	+	+	+	+	+
Cys-146	−	−	−	−	−	+	±	−	−	−	−	−	−
His-147	+	+	+	−	±	+	+	−	±	+	−	+	−
Gln-151	−	−	∓	−	−	∓	−	∓	+	+	−	−	+
Arg-166	∓	−	−	−	−	−	−	−	−	∓	−	+	−
Asp-169	−	−	−	−	−	±	−	−	−	−	−	−	−
Asn-177	±	−	−	−	±	−	±	−	±	−	−	∓	−
Gly-204	+	+	−	−	−	−	±	−	−	−	−	−	−
Glu-223	+	+	+	+	+	+	+	+	+	+	+	+	+

Purified colonies of the 20 *thyA* amber mutants transformed into 13 amber suppressor strains were streaked onto media with or without thymidine, and growth of individual colonies was compared at 37°C. +, 50–100% of wild type; ±, 10–50%; ∓, <10%; −, no individual colonies observed.

Table 2. Amino acid substitution by suppression or missense

Site	Amino acid substitution	Growth characteristics	
		Suppression	Missense
His-147	Ala	+	+
	Glu	–	±
Asp-169	His	–	–
	Glu	–	±

Four of the variants created by suppression of amber nonsense mutations were made as missenses, and their growth characteristics were compared as described in the legend to Table 1.

Missense Mutations. We constructed missense mutations of four of the variants in order to determine if amino acid substitution by suppression of amber nonsense mutations correlated to missense data. Two of the missense mutations had growth characteristics that matched the corresponding suppressed variant, whereas the other two missense mutations grew slightly better as missenses (Table 2). In general, missense mutations would be expected to grow as well as or better than their suppressed counterparts because the efficiency of suppression can range from 5% to 100%, depending upon the suppressor and the surrounding context (18). Thus, plasmids bearing missense mutations can sometimes over-come the effect of the mutation by overproduction of the mutant enzyme.

Enzyme Assays. We assayed extracts of selected mutants as described in *Materials and Methods*. The results are shown in Table 3. Several points can be made from these assays. First, the total activity values of thymidylate synthase variants in the crude extract show that (*i*) + variants can have activities as low as 2.68% of the activity of the wild-type extract, (*ii*) a ± (Leu-147) variant has 0.72% of the activity of the wild-type extract, and (*iii*) – variants have ≤0.13% of the activity of the wild-type extract. Second, the specific activity values of thymidylate synthase variants show that (*i*) + variants can have specific activities as low as 1.25% of the wild-type enzyme, (*ii*) a ± (Leu-147) variant has a specific activity that is 0.97% of the wild-type enzyme, and (*iii*) – variants have specific activities that are ≤0.32% of the wild-type enzyme.

DISCUSSION

We have used site-directed oligonucleotide mutagenesis to introduce amber nonsense codons (5'-TAG-3') at 20 sites in the *E. coli* thymidylate synthase gene (Fig. 1) and have made 12 or 13 amino acid substitutions at each site by transforming

Table 3. Activities of suppressed thymidylate synthase variants

Wild-type amino acid*	Substitution†	Growth‡	Proposed function§	Total activity,¶ units	TS,‖ μg/ml	Specific activity,** units	% wild-type activity††
Phe-30	Ala	–	Role in	0.80 ± 0.73	5.12 ± 1.75	0.16 ± 0.20	0.32
	Phe	+	tertiary	973.52 ± 142.38	32.51 ± 1.80	23.36 ± 4.71	46.46
	Ser	–	structure of	0.37 ± 0.13	4.96 ± 1.11	0.08 ± 0.05	0.16
	Tyr	+	the binding	77.03 ± 17.26	1.88 ± 0.59	42.58 ± 22.90	84.69
	Leu	+	pocket	246.82 ± 18.46	3.94 ± 0.73	61.51 ± 16.00	122.33
Gln-33	Ala	+	No role in	38.28 ± 5.74	1.16 ± 0.04	26.09 ± 4.81	51.89
	Phe	+	catalysis	409.02 ± 169.86	6.06 ± 2.22	36.35 ± 28.41	72.30
	Ser	+	(conserved	519.72 ± 39.88	7.12 ± 0.71	49.72 ± 8.78	98.89
	Tyr	+	amino acid	485.25 ± 16.25	16.34 ± 10.44	48.67 ± 32.72	96.80
	Leu	+	among all	675.62 ± 63.32	31.30 ± 8.92	22.26 ± 8.43	49.27
	Gln	+	known TSs)	602.52 ± 54.22	16.15 ± 0.91	49.25 ± 7.21	97.95
Arg-127	Ala	+	Role in dUMP	27.56 ± 14.12	0.69 ± 0.02	28.78 ± 15.63	57.24
	Phe	+	binding	54.10 ± 1.56	10.51 ± 2.37	4.61 ± 1.17	9.17
	Ser	+		54.94 ± 5.27	4.22 ± 0.35	10.60 ± 1.90	21.08
	Tyr	+		48.93 ± 8.42	8.76 ± 1.19	8.48 ± 2.61	16.86
	Leu	+		550.26 ± 77.75	27.74 ± 5.58	20.05 ± 6.87	39.88
	Arg	+		361.54 ± 21.40	13.24 ± 2.29	31.82 ± 7.39	63.29
His-147	Ala	+	Role during	93.01 ± 11.82	38.43 ± 3.31	3.45 ± 0.74	6.86
	Phe	–	catalysis	0.73 ± 0.03	49.30 ± 3.11	0.03 ± 0.01	0.06
	Ser	+		21.19 ± 1.67	57.18 ± 3.51	0.63 ± 0.09	1.25
	Tyr	–		1.02 ± 1.70	9.96 ± 3.26	0.12 ± 0.20	0.24
	Leu	±		5.71 ± 1.97	19.14 ± 2.96	0.49 ± 0.24	0.97
	His	+		1436.61 ± 23.89	46.69 ± 4.40	43.08 ± 4.78	85.68
Wild-type plasmid	None	+		790.05 ± 151.35	26.40 ± 7.22	50.28 ± 23.38	100.00

TS, thymidylate synthase.
*Site of amber suppression and the original wild-type amino acid at that position in *E. coli* thymidylate synthase.
†Amino acid inserted at the amber codon.
‡Growth phenotype as described in Table 1.
§Proposed role of the amino acid during catalysis as determined by the crystal structure.
¶Activity of thymidylate synthase enzyme with respect to total extract protein. Values are the means ± SD of the tritium-release assays for two or more independent determinations. One unit is defined as the release of 1 nmol of tritium per hr per mg of total extract protein under the conditions described in *Materials and Methods*.
‖Concentration of thymidylate synthase in the extract as measured by quantitative Western blotting. Values are the means ± SD of the densitometry values for two or more independent determinations.
**Specific activity of suppressed variants where the concentration of thymidylate synthase in the extract was determined by quantitative Western blots. Values represent the means ± the combined standard deviation relative to standard deviations for the total activity and the thymidylate synthase concentration. One unit is defined as the release of 1 μmol of tritium per hr per mg of thymidylate synthase enzyme under the conditions described in *Materials and Methods*.
††Percent of wild-type activity where wild-type activity was determined from an extract prepared from *E. coli* containing the wild-type *thyA* gene on the pACYC 184 vector. The concentration of wild-type thymidylate synthase in the extract was determined by quantitative Western blots.

the mutants into suppressor strains. The suppressor strains have altered tRNAs that can insert an amino acid in response to an amber stop codon. In this way, we have created over 245 variants of thymidylate synthase.

The *thyA* mutants are on a low-copy-number plasmid, pACYC 184, which typically maintains 20 copies per cell (20). Suppression of the amber mutation is commonly 25–50% efficient (18), resulting in roughly 5–10 copies of the variant in each cell. The low copy number allows the system to be relatively sensitive to mutations that weaken but do not abolish enzyme activity. If such variants were present at high copy number, they might restore the cell to Thy$^+$ by the overall activity of the overproduced mutant enzyme.

Growth characteristics of the variants are presented in Table 1, and enzyme assays of selected variants are shown in Table 3. Four of the amber sites that we probed were predicted to be innocuous based upon their exposed surface location in the recently published crystal structure of a ternary complex of *E. coli* thymidylate synthase with two bound inhibitors, FdUMP and 10-propargyl-5,8-dide-azafolate (Fig. 2). These sites (Glu-14, Asp-105, Asn-121, and Glu-223) were substitutable by all 13 amino acids, regardless of the surrounding nucleotide context (Table 1; ref. 28). Another set of four amber sites also proved to be highly substitutable, even though all are either absolutely or very highly conserved among the known thymidylate synthases (Gln-33, Arg-35, Asp-81, and Arg-127) (29). The remaining 12 sites would only accept limited substitutions and include residues that form parts of the substrate binding pockets, the active site nucleophile, or important structural elements in thymidylate synthase (5, 6, 29).

The activity of some of the substitutions was surprising in light of their proposed roles based upon the recently published crystal structure of a ternary complex of *E. coli* thymidylate synthase–FdUMP–10-propargyl-5,8-dideaza-folate (5, 6) and a previously published crystal structure of *Lactobacillus casei* thymidylate synthase solved without bound substrates or inhibitors (29). For example, both structures suggest that Arg-127 plays a role in binding the phosphate group of dUMP, yet it was substitutable by all 13 amino acids with retention of 10–50% of the wild-type activity (Table 3), suggesting that it is not important for PO$_4^{2-}$ binding. However, three other Arg residues that were proposed to interact with the phosphate group (Arg-21, Arg-126, and Arg-166) would only accept limited substitutions. Among the three Arg residues, there appears to be a hierarchy of substitutability. Arg-126 can be replaced by any basic amino acid as well as by Tyr, but Arg-21 and Arg-166 are only restored to Thy$^+$ by their cognate amino acid.

Another interesting substitution pattern resulted at Asp-110, which forms a salt bridge with Arg-99 and thereby aligns helixes IV and V in an extended kinked helix (29). Activity could be partially restored by substitution of Cys or Ser for Arg, but surprisingly it was not restored by Glu. One could argue that the extra methylene group in Glu poses a steric hindrance; however, Tyr, which is far more bulky, also partially restored activity. On the basis of our analysis of the x-ray structure, a more likely explanation for this observation is that replacement of Asp with Glu requires the longer glutamate side chain to fold into a high energy conformation in order to maintain the important charge-mediated hydrogen-bonding interaction with the guanidinium group of Arg-99. Comparison of the crystal structure of these two mutants could help define the role that this salt bridge plays in maintaining the overall structure of thymidylate synthase.

A unique structural feature of thymidylate synthase is that the central β-sheets of the dimer are related by a right-handed rather than a left-handed twist (5). This structure is the direct result of an unusual series of stacked β-bulges that begin at the protein surface and extended 20 Å down the middle of the
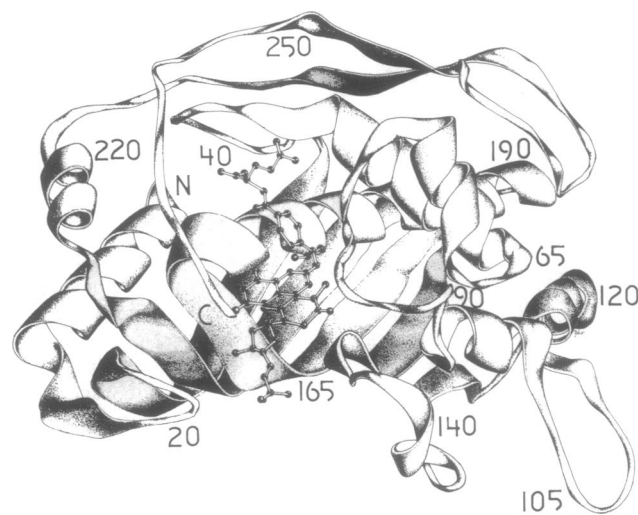

FIG. 2. (*Upper*) Representation of backbone chain folding for one subunit of *E. coli* thymidylate synthase containing bound FdUMP (yellow) and 10-propargyl-5,8-dideazafolate (red). Each of the 20 sites targeted for substitution in this study is indicated by a ball and stick representation of α carbon and side chain atom positions for the corresponding residue in the wild-type *E. coli* enzyme. The color code is as follows: Glu and Asp, red; Asn, Gln, His, Cys, and Ser, light blue; Phe and Trp, green; Arg, dark blue; Gly, gray. Two of the four Arg residues interacting with bound FdUMP (residues 126 and 127) come from the other subunit of the thymidylate synthase dimer, and their α carbon atoms are colored gray. The covalent bond connecting the active site sulfur of Cys-146 with C-6 of FdUMP is indicated in red. (*Lower*) For clarity, amino acid numbering is indicated in black and white.

central β-sheet, ultimately accounting for the positioning of residues important for substrate binding and the precise orientation of the catalytic Cys-146. Phe-30, Gln-151, and Gly-204 contribute hydrogen bonds that may be important for maintaining this unusual structure, and they only accept a few substitutions. For example, Tyr-30 can be substituted for Phe-30 without diminishing enzyme activity (Table 3). Unexpectedly, the amino acid at this position could also be replaced with leucine, but all other substitutions rendered the enzyme inactive (Table 1 and Table 3). Surprisingly, Arg-35, a conserved surface residue whose side chain forms hydrogen bonds with the backbone carbonyls of Phe-30 and Gly-31 and

thereby may stabilize the stacked β-bulges, was nearly completely substitutable. The dihedral angles necessary to generate the nearly parallel alignment of the carbonyl atoms of residues 203 and 204 are predicted to be forbidden for any side chain larger than hydrogen at position 204. However, we observe that Ala can be substituted at this position. It will be interesting to determine how this substitution affects the structure of the stacked β-bulges and influences kinetic parameters.

His-147 lies in the binding pocket of thymidylate synthase and is conserved in all known thymidylate synthases except in phi3T phage where it is a Val. It has been recently suggested that His-147 acts indirectly during catalysis by protecting the protonated form of a catalytically important Lys or Arg residue (30). The protonated form of such a Lys or Arg residue is probably required for folate cofactor binding (30). Replacement of His-147 with amino acids such as Ala, Ser, and Leu diminishes enzyme activity but does not inactivate the enzyme. Replacement with amino acids with bulkier side chains such as Phe and Tyr, as well as Lys, renders the enzyme inactive.

Other sites in the protein were substituted more predictably. For instance, Trp-80, Asp-169, and Asn-177, which form contacts with both dUMP and 5,10-methylenetetrahydrofolate, could not be fully restored to Thy$^+$ by any of the substitutions we made. These residues are strictly conserved in all known thymidylate synthase enzymes.

The active site nucleophile, Cys-146, only accepted its original amino acid. However, partial activity was restored with the Ser substitution. This correlates well with previous missense mutation studies at position 146. Dev *et al.* (31) reported that substitution of Ala, Gly, or Thr at position 146 results in an inactive enzyme but that a Ser mutant retains a small amount of catalytic activity.

The technique of using suppressors to introduce amino acid substitutions allows one to rapidly determine the effect of a wide variety of amino acid substitutions.

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