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Cysteinyl-tRNA Synthetase from *Escherichia coli* Does Not Need an Editing Mechanism to Reject Serine and Alanine. High Binding Energy of Small Groups in Specific Molecular Interactions†

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ABSTRACT: The cysteinyl-tRNA synthetase from *Escherichia coli* only very slowly activates serine, alanine, and α-aminobutyrate, the possible competitors of cysteine. The upper limits on the values of k_{cat}/K_M for the amino acid dependent ATP/pyrophosphate exchange reactions, relative to that of cysteine, are $<10^{-8}$, 2×10^{-7}, and 3×10^{-6}, respectively. It is calculated from these data and the concentrations of the amino acids in vivo that the error rates for the misincorporation of serine and alanine for cysteine are less than 10^{-9} and 5×10^{-8}, respectively. There is no need for an error correcting mechanism and no evidence has been found to implicate one: there is no detectable ATP/pyrophosphatase activity of the enzyme in the presence of $tRNA^{Cys}$ and alanine; Ala-$tRNA^{Cys}$ has been synthesized by the reductive desulfurization of Cys-$tRNA^{Cys}$ and has been found to be relatively resistant to the enzyme-catalyzed deacylation. Part of the high selectivity of the enzyme for the –SH group of cysteine (~5 kcal/mol) appears to be caused by dispersion forces: simple calculations suggest that the dispersion energy between sulfur and a methylene group is about 2.5 times greater than that between two methylene groups. This high "hydrophobicity" of sulfur is consistent with the relative binding energies of substrates of the methionyl-tRNA synthetase. The rest of the high binding energy of the –SH group may come from hydrogen bonding.

Not all aminoacyl-tRNA synthetases are faced with severe problems in rejecting competing amino acids. Certain amino acids are sufficiently different in structure from their nearest competitors that adequate specificity may be provided by simple binding. Phenylalanine, for example, binds at least 3×10^4 times more weakly than tyrosine to the tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* (Fersht et al., 1975) because phenylalanine lacks the hydrogen bonding potential of the phenolic hydroxyl. In this context, it is noted that the tyrosyl-tRNA synthetase is one of the few aminoacyl-tRNA synthetases that will aminoacylate either the 2′- or 3′-terminal hydroxyl groups of the cognate tRNA (Cramer et al., 1975). The valyl- and isoleucyl-tRNA synthetases are specific for the 2′ hydroxyl in aminoacylation (Cramer et al., 1975; Sprinzl & Cramer, 1975; Chinault et al., 1976) but require the nonaccepting 3′ hydroxyl in some way for the hydrolytic editing (von der Haar & Cramer, 1975; Igloi et al., 1977). It has been suggested that the aminoacylation and editing sites are on either side of the two terminal hydroxyl groups (Fersht & Kaethner, 1976). The lack of specificity for the 2′- or 3′-hydroxyl group would be consistent with the notion that the tyrosyl-tRNA synthetase also lacks a hydrolytic site for the deacylation of mischarged tRNA.

The cysteinyl-tRNA synthetase is another example that will aminoacylate both the 2′- and 3′-hydroxyl groups. There is the experimental advantage with this enzyme that the sulfur of its correctly charged cognate tRNA may be removed reductively with Raney nickel to produce the mischarged species, Ala-$tRNA^{Cys}$ (Chapeville et al., 1962). Alanine, being smaller than cysteine by one sulfur atom, is one of the most likely competitors of cysteine for the active site of the cysteinyl-tRNA synthetase.

In this paper, we have determined the specificity of the cysteinyl-tRNA synthesis in the pyrophosphate exchange reaction for cysteine, alanine, serine and α-aminobutyrate to determine whether adequate selection is possible without a subsequent deacylating mechanism. We have prepared Ala-$tRNA^{Cys}$ to see whether it is rapidly deacylated by the enzyme in a possible error correcting step.

Experimental Section

Materials. The cysteinyl-tRNA synthetase was isolated from *Escherichia coli* as described elsewhere (Mulvey et al., 1978). Partially purified $tRNA^{Cys}$ (amino acid acceptance = 30 pmol/A_{260} unit) was obtained from the Microbiological Research Establishment, Porton Down, Wiltshire, England. Radioactively labeled amino acids were obtained from the Radiochemical Centre, Amersham, England. Unlabeled amino acids were obtained from Sigma or BDH and further purified by recrystallization from ethanol–water.

The concentration of the cysteinyl-tRNA synthetase was calculated using a mol wt of 59000 and a value of 2 cm^{-1} mg^{-1} mL^{-1} for A_{280} (Mulvey et al., 1978). Activity was routinely checked by active site titration (Fersht et al., 1975), and purity was evaluated by polyacrylamide gel electrophoresis using sodium dodecyl sulfate.

Preparation of [^3H]*Cys-tRNA*^{Cys}. A solution (0.68 mL) of [3,3′-^{3}H]cystine (74 μM, 1.6 Ci/mmol), Tris-Cl (pH 7.8, 0.64 M), and dithiothreitol (44 mM) was incubated at 25 °C to reduce the cystine to cysteine. After 15 min, tRNA (250 A_{260} units/mL), $MgCl_2$ (10 mM), ATP (2 mM), phenylmethanesulfonyl fluoride (0.1 mM), inorganic pyrophosphatase (0.3 units/mL), and cysteinyl-tRNA synthetase (0.5 μM) were added to give a total volume of 3 mL. After a further 10 min, the pH was lowered to 5 by the addition of sodium acetate buffer (0.2 M) and the solution extracted with an equal volume

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Table I: Amino Acid Dependent Pyrophosphate Exchange Reactions of the Cysteinyl-tRNA Synthetase[a]

amino acid (mM)	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1} M^{-1})	rel values of k_{cat}/K_M	$\Delta\Delta G^b$ (kcal mol^{-1})
Cys (0.01–2)	22	0.05	4.4×10^5	1	0
Ser (550)			$<4 \times 10^{-3}$	$<10^{-8}$	>11
α-aminobutyrate (75–180)	>0.2	>180[c]	1.29	2.9×10^{-6}	≥7.6
Ala (125–365)	>0.03	>365[c]	0.088	2×10^{-7}	≥9.1

[a] 25 °C, pH 7.78, 10 mM $MgCl_2$, 2 mM [^{32}P]pyrophosphate, 2 mM ATP, 0.1 mM phenylmethanesulfonyl fluoride, and 14 mM β-mercaptoethanol. Cysteinyl-tRNA synthetase (1.2 μM) was used except for the reactions with cysteine (37 nM). [b] Relative binding energies calculated from k_{cat}/K_M. [c] No detection of saturation kinetics at highest concentration of substrate used.

of saturated phenol. The tRNA was precipitated from the aqueous layer by the addition of 2 volumes of ethanol and cooling to −30 °C. The precipitate was dissolved in 10 mM $MgCl_2$ and desalted on Sephadex G-25 equilibrated with 10 mM $MgCl_2$ (~pH 5).

Reductive Desulfurization of [^{3}H]Cys-tRNA^{Cys}. A solution (12 mL) containing [^{3}H]Cys-tRNA (650 A_{260} units), 0.1 mL of saturated EDTA, and 640 mg of Raney nickel (prepared by the method of Vogel, 1967) in sodium citrate buffer (pH 4.5, 0.75 M) was incubated with agitation at 25 °C for either 2.5 or 6 h. The nickel was removed by centrifugation and the supernatant dialyzed overnight against citrate (pH 4.5, 100 mM), $MgCl_2$ (10 mM), and EDTA (2.5 mM). After dialyzing for a further 24 h against 10 mM $MgCl_2$ and EDTA (disodium salt, pH 4.6, 2.5 mM), the tRNA was precipitated by the addition of 2 volumes of ethanol and gel filtered on Sephadex G-25 into 10 mM $MgCl_2$. A parallel experiment was performed to monitor the desulfurization by using [^{35}S]Cys-tRNA^{Cys} (120 Ci/mol) under identical conditions.

The amino acid was removed for analytical purposes by incubating with sodium phosphate (pH 11, 33 mM) at 25 °C for 1 h. A control was set up by the addition of an authentic sample of [^{3}H]Ala to [^{35}S]Cys-tRNA incubated under the same conditions. The two samples were then subjected to high voltage electrophoresis (pH 2.1, acetic acid/formic acid buffer, Whatman no. 1 paper, 40 min at 3 kV).

To test the integrity of the tRNA after reduction, a sample was deacylated by incubating with 0.3 M Tris-Cl (pH 7.8) at 37 °C for 90 min and then aminoacylated with [^{35}S]Cys.

Standard Conditions for Kinetic Experiments. All experiments were performed at 25 ± 0.1 °C in buffers containing 10 mM $MgCl_2$, 14 mM mercaptoethanol, 0.1 mM phenylmethanesulfonyl fluoride, and either 144 mM Tris-Cl at pH 7.78, or, where indicated, 13 mM Bistris-Cl at pH 5.87.

Pyrophosphate exchange and ATP/pyrophosphatase activities were measured as described by Fersht & Kaethner (1976) and Fersht (1977b).

The cysteinyl-tRNA synthetase catalyzed deacylation of Cys-tRNA^{Cys} and Ala-tRNA^{Cys} was measured in the presence of 2.5 mM EDTA to inhibit any possible Ni^{II} catalyzed deacylation. An excess of enzyme over tRNA was used as described previously (Fersht & Dingwall, 1979). The concentration of *free* Mg^{2+} in these experiments was adjusted to 15 mM; the tRNA was preequilibrated with $MgCl_2$ to saturate its Mg^{2+} binding sites. Reaction volumes of 100 μL were used and 15-μL samples were periodically quenched with trichloroacetic acid.

Results

(*a*) *Pyrophosphate Exchange Reactions.* The kinetic data for the pyrophosphate exchange reactions are listed in Table I. Appreciable activity is found only in the presence of cysteine. There is no inhibition by the mercaptoethanol in the buffer; lowering the concentration 30-fold does not affect k_{cat} or K_M.

There is extremely low, but real, activity in the presence of alanine and α-aminobutyrate. This remained unchanged on recrystallization of the amino acids. Saturation kinetics could not be observed and the rate increased linearly with concentration of amino acid. The values of k_{cat}/K_M were calculated from this activity.

Some activity is just detectable in the presence of serine at 0.55 M: pyrophosphate is exchanged into ATP at the rate of 2.2×10^{-3} mol per mol of enzyme per s, 0.01% of that with cysteine under the same conditions.

The low activity with, for example, alanine is certainly not caused by the presence of trace amounts of the alanyl-tRNA synthetase since this would involve a low value for the K_M for the amino acid. There is the possibility that the presence of a noncognate aminoacyl-tRNA synthetase, such as the valyl-tRNA synthetase, could be catalyzing these reactions, since its misactivations also involve high values of K_M. In view of this, the data must be considered as *upper* limits of the true activity.

(*b*) *ATP/Pyrophosphatase Activity.* There is negligible activity at the highest concentration of alanine used. In the presence of 1.4 μM tRNA^{Cys}, 1.1 μM cysteinyl-tRNA synthetase, 2 mM [γ-^{32}P]ATP, 1 unit of inorganic pyrophosphatase/mL, and 0.5 M alanine, in the standard pH 7.78 buffer, the ATP is hydrolyzed at 2.8×10^{-3} mol per mol of enzyme per s compared with 3.6×10^{-3} in the absence of alanine. Under these conditions, but substituting 2 mM pyrophosphate for the inorganic pyrophosphatase, pyrophosphate exchange takes place at 4.4×10^{-2} mol exchanged per mol of enzyme per s.

(*c*) *Reductive Desulfurization of Cys-tRNA^{Cys}.* The original procedure of Chapeville et al. (1962) for the reductive desulfurization proved unsatisfactory for our purpose. They found that after 30 min incubation of Cys-tRNA^{Cys} with Raney nickel in 0.4 M sodium acetate buffer at pH 5 and EDTA, 50% of the aminoacyl-tRNA was deacylated and only 30–70% of the cysteine reduced. Ni^{II} ions are known to catalyze the hydrolysis of amino acid esters and so the acetate buffer was replaced by citrate buffer, which is a good chelating agent for Ni^{II} at low pH (EDTA is a relatively poor chelating agent at low pH because of its low solubility and unfavorable ionization state).

The results of small scale pilot runs are given in Figure 1. Using [^{35}S]Cys-tRNA^{Cys} to monitor the loss of ^{35}S, it is seen that after 2.5 h about 75% is desulfurized, while 90% of (initially) [^{3}H]Cys-tRNA^{Cys} still remains acylated. The preparative reductive desulfurization gave after 2.5 h of incubation aminoacyl-tRNA charged to 25 pmol/A_{260} (83% of initial 30 pmol/A_{260}). Analysis of the attached amino acid showed that 60% is [^{3}H]Ala and 40% probably unreacted cysteine. After mild deacylation, the tRNA would accept 25 pmol/A_{260} of [^{35}S]Cys. After 6-h incubation with Raney nickel, the isolated aminoacyl-tRNA is charged to 15.5 pmol/A_{260} (50%) and 74% of the attached amino acid is [^{3}H]Ala and the remaining 26% probably unreacted cysteine.

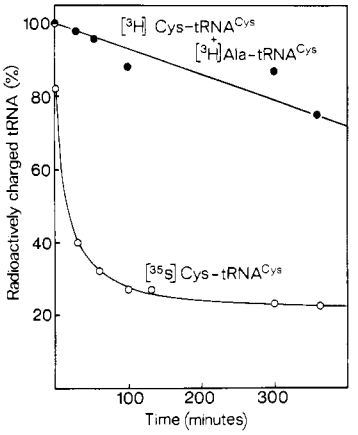

FIGURE 1: Raney nickel catalyzed desulfurization and deacylation of Cys-tRNA^{Cys} (see text). Deacylation measured by loss of acid-precipitable radioactivity from [^{3}H]Cys-tRNA^{Cys}; desulfurization and deacylation were monitored by loss of [^{35}S]Cys-tRNA^{Cys}.

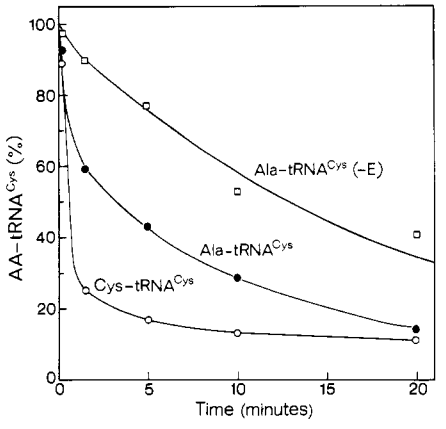

FIGURE 2: Deacylation of Ala-tRNA^{Cys} and Cys-tRNA^{Cys} at pH 7.78, 25 °C, and standard conditions. (□) [^{3}H]Ala-tRNA^{Cys}, 1.4 μM; (●) 1.4 μM [^{3}H]Ala-tRNA^{Cys} + 2.2 μM cysteinyl-tRNA synthetase; (○) 1.4 μM [^{3}H]Cys-tRNA^{Cys} + 2.2 μM cysteinyl-tRNA synthetase.

The tRNA could be recharged to 18 pmol/A_{260} after deacylation. Both preparations of mischarged tRNA were used in the deacylation experiments.

The yield of alanine is similar to that reported by Perlstein et al. (1971) for the reductive desulfurization of cystine and cysteine at pH 5 and 40 °C. They obtained 70% alanine and 30% an unknown amino acid.

(d) Deacylation of Cys-tRNA^{Cys} and Ala-tRNA^{Cys}. The hydrolytic data are presented in Figures 2 and 3. At pH 7.78, the Ala-tRNA^{Cys} deacylates with a first-order rate constant of about 10^{-3} s^{-1}. Addition of excess cysteinyl-tRNA synthetase increases the hydrolysis rate by a small factor but the kinetics are no longer first order. The rate constant calculated from the initial rate of hydrolysis of Ala-tRNA^{Cys} is about 4×10^{-3} s^{-1}. The initial rate constant for the enzyme-catalyzed deacylation of Cys-tRNA^{Cys} is initially about five times faster at 0.02 s^{-1}. At pH 5.87, all three rate constants are decreased tenfold. The data presented in Figure 2 are for the tRNA which was incubated for 6 h with Raney nickel.

Discussion

The basic problem in achieving high enzyme specificity is the rejection of substrates that are smaller than or isosteric with the correct substrate. The most likely competitors of cysteine for the cysteinyl-tRNA synthetase are amino acids with an unbranched side chain the same length as or shorter than that of cysteine. It is seen in Table I that the competitive

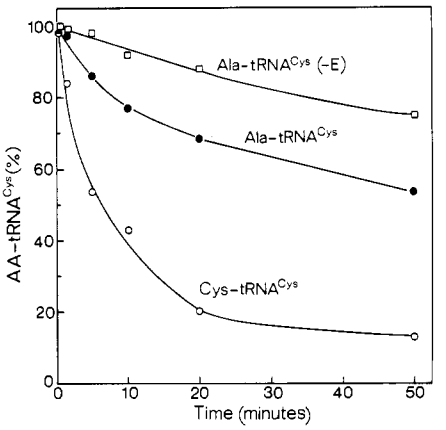

FIGURE 3: Deacylation of Ala-tRNA^{Cys} and Cys-tRNA^{Cys} at pH 5.87, 25 °C, and standard conditions. (□) [^{3}H]Ala-tRNA^{Cys}, 1.4 μM; (●) 1.4 μM [^{3}H]Ala-tRNA^{Cys} + 2.6 μM cysteinyl-tRNA synthetase; (○) 1.4 mM [^{3}H]Cys-tRNA^{Cys} + 2.2 μM cysteinyl-tRNA synthetase.

amino acids are such poor substrates for the pyrophosphate exchange reaction that, even without a correction mechanism, misincorporation would be very low. The relative rates of reaction of two substrates A and B in a mixture of the two are given by (Fersht, 1977b)

$$v_A/v_B = (k_{cat}/K_M)_A[A]/(k_{cat}/K_M)_B[B] \quad (1)$$

Using the rate constants for the pyrophosphate exchange reaction (Table I) and the known intracellular concentrations of alanine and serine (Raunio & Rosenqvist, 1970), it may be calculated that, if all the alanine and serine activated by the enzyme is incorporated into proteins, the error rate for the misincorporation of alanine for cysteine would be 5×10^{-8}, and that of serine for cysteine would be less than 10^{-9}. The misincorporation of alanine and serine for cysteine may well be lower than that calculated from the data in Table I since these are upper limits of the true activity of the cysteinyl-tRNA synthetase. These are far lower than the experimentally observed error rate of 3×10^{-4} for the misincorporations of valine for isoleucine that survive to be measured in rabbit globin (Loftfield & Vanderjagt, 1972). An editing mechanism is known to operate for these two amino acids in *E. coli*.

There is thus no need for an error correcting mechanism at the level of aminoacylation and there is no evidence that one exists. We have found no detectable ATP/pyrophosphatase activity of the enzyme in the presence of alanine and tRNA^{Cys}. This activity has been found, however, in all cases where editing has been shown to occur, since ATP is consumed when aminoacyl adenylate is formed and then directly or indirectly hydrolyzed. Our data also suggest that it is extremely unlikely that there is any editing by the deacylation of mischarged tRNA. Ala-tRNA^{Cys} is five times more stable than Cys-tRNA^{Cys} in the presence of cysteinyl-tRNA synthetase. It could perhaps be argued that the treatment of Cys-tRNA^{Cys} with Raney nickel destroys an essential recognition element of the tRNA. However, we have shown that the Ala-tRNA^{Cys} may be recharged with cysteine after deacylation, and, after similar treatment with Raney nickel, the alanyl moiety of Ala-tRNA^{Cys} may still be incorporated into proteins (Chapeville et al., 1962).

Physicochemical Origin of the High Specificity for Cysteine. The binding energies of groups in the side chains of amino acids may be calculated from the relative values of k_{cat}/K_M for the activation reaction (Fersht, 1977b, pp 234, 276). It is seen in Table I that the sulfur atom in cysteine contributes greater than 9 kcal/mol of binding energy (relative to its absence in alanine). There is also at least 7.6 kcal/mol relative

Table II: Calculated Relative Dispersion Energies of -O-, $-CH_2-$, and -S- with the $-CH_2-$Group

atom/group	polarizability[a] (mL × 10^{24})	van der Waals radius (Å)	rel interaction energy with $-CH_2-$ (kcal mol^{-1})[b]
-O-	0.63	1.5	1
$-CH_2-$	1.80	2.0	1
-S-	3.00[c]	1.8	2.5

[a] Calculated from R_∞ extrapolated from Le Fevre (1965, Table V). [b] Atoms separated by sum of van der Waals' radii. "6-12" function and modified Slater-Kirkwood equation used. [c] This is a factor of ten higher than that listed by Gibson and Scheraga (1967) and Scheraga (1968).

to the $-CH_2-$ group in α-aminobutyrate, and greater than 11 kcal/mol relative to the -O- in serine. These values are far higher than might have been expected.

(a) Dispersion Forces. It has been shown that, in a comparison of the binding of valine and isoleucine to the isoleucyl-tRNA synthetase and α-aminobutyrate and valine to the valyl-tRNA synthetase, the binding energy of the additional $-CH_2-$ group on the correct substrates contributes a factor of 2.9–3.4 kcal/mol. This energy, which is far higher than the usual value of about 0.6–0.7 kcal/mol normally found for hydrophobic bonding, arises mainly from the high dispersion or van der Waals' forces between the enzyme and substrate (Jencks, 1975; Page, 1976). Dispersion energies increase both with increasing polarizability of atoms and the number of atoms involved since the energies are additive. The forces are particularly weak in water because of the low polarizability of oxygen and the low number of atoms per unit volume. These forces can be much higher, however, in an enzyme–substrate interaction where the enzyme has evolved to close-pack its atoms around a group on the substrate. The dispersion energy between a methylene group and its neighbor in a solid hydrocarbon is 2 kcal/mol (measured from heats of sublimation, M. Levitt & S. Lifson, quoted in Fersht, 1977b, Chapter 9). The energy in a protein is probably similar (Page, 1976).

We have calculated the dispersion energies for the interaction of -S-, $-CH_2-$ and -O- with a $-CH_2-$ group to estimate the relative contributions of the side chains in the binding of cysteine, α-aminobutyrate, and serine to the cysteinyl-tRNA synthetase. This was done by the procedure described by Gibson & Scheraga (1967) and Scheraga (1968): (a) the polarizabilities of -O-, -S-, and $-CH_2-$ were calculated from the molecular refractivities (extrapolated to infinite wavelength) listed by Le Fevre (1965); (b) the attractive potential was calculated according to the Slater–Kirkwood equation using the "effective" number of electrons listed by Scheraga (1968) and treating $-CH_2-$ as a single atom (Gibson & Scheraga, 1967); (c) the interaction energy was calculated assuming a "6–12" function with the atoms being separated by the sums of their van der Waals' radii. The results, listed in Table II, are probably accurate to only a factor of three or so in absolute terms, but we believe their *relative* values to be significant. The dispersion forces between -S- and $-CH_2-$ are 2.5 times greater than between $-CH_2-$ and $-CH_2-$. Thus the dispersion energy between -S- and a hydrocarbon environment is 2.5 × 2 kcal/mol = 5 kcal/mol. This figure appears to be consistent with experiments comparing the binding of norleucine (side chain = $CH_3(CH_2)_3-$) and methionine (R = $CH_3S(CH_2)_2-$) and norvaline (R = $CH_3(CH_2)_2-$) and homocysteine (R = $HS(CH_2)_2-$) to the methionyl-tRNA synthetase from *B. stearothermophilus* (Fersht & Dingwall, 1979b). In both cases, comparison of the values of k_{cat}/K_M for the pyrophosphate exchange reaction shows that the substitution of -S- for $-CH_2$ increases the binding energy by 3.14 kcal/mol. Similarly, comparison of the data for the activation of α-aminobutyrate and homocysteine leads directly to a figure of 5.4 kcal/mol for the binding energy of sulfur to the methionyl-tRNA synthetase.

These data show that -S- is more "hydrophobic" than $-CH_2-$ because of stronger dispersion forces and indicate that the sulfur atom contributes about 5 kcal/mol to binding through this effect.

(b) Chemical Nature of the Thiol Group. As the 5 kcal/mol or so of dispersion energy is inadequate to account for the high selectivity indicated in Table I, the cysteinyl-tRNA synthetase must also use other binding properties of the thiol group of cysteine. There is now good evidence that the -SH group is a hydrogen bond donor/acceptor (Crampton, 1974; Paul, 1974). The presence of hydrogen bond donor/acceptor sites on the enzyme could account for the relatively weak binding of alanine and α-aminobutyrate. However, as the hydroxyl group of serine forms far stronger hydrogen bonds than the thiol group (Crampton, 1974), it might be argued that serine should bind strongly to the cysteinyl-tRNA synthetase. But, in hydrogen bonding to the enzyme, the substrate sheds its hydrogen bonds to water. The contribution of hydrogen bonding to the binding energy of the enzyme–substrate complex thus depends on the *difference* in hydrogen bonding energies with the enzyme and solvent. It is perhaps possible that the geometry of serine (C–O bond distance = 1.43 Å, O–H = 0.88 Å, ∠ C–O–H = 107°; Kistenmacher et al., 1974) is sufficiently different from that of cysteine (C–S = 1.8 Å, S–H = 1.4 Å, ∠ C–S–H = 93°; Kerr et al., 1975) that hydrogen bonding of the enzyme with serine is unfavorable. In this case, the strong hydrogen bonding of serine with water would actually depress binding to the enzyme.

The current feeling is that the intramolecular hydrogen bonding between an enzyme and a substrate is entropy driven, as bound water molecules are freed (Jencks, 1975). The release of two bound water molecules could contribute some 5 kcal/mol at 25 °C (Fersht, 1977b, p 273). The combined dispersion energy and hydrogen bonding interactions of the -SH group may thus possibly contribute up to 10 or so kcal/mol of binding energy.

There are two other possibilities that are unique to sulfur. First, thiols have good chelating properties. For example, cysteine binds Zn^{2+} some 10^5 times more tightly than does serine (Perrin & Sayce, 1968; Sychev, 1964) by using the combined $-S^-$ and $-NH_2$ groups (Friedman, 1973). Our evidence speaks against cysteine binding as a thiolate anion to a metalloion in the synthetase, since this would predict that binding should weaken at low pH as the thiol group becomes less ionized. We find that the K_M for the pyrophosphate exchange reaction at pH 5.87 is about half that at pH 7.78, whilst k_{cat} remains unchanged. Second, there is the possibility of a rapid and reversible thiol exchange reaction with a -S–S- cross-bridge on the enzyme to form a temporary covalent link between the cysteine substrate and a cysteinyl residue. There is weak evidence against this possibility in that all our attempts to isolate a denatured covalent complex between the enzyme and cysteine failed.

On balance, we feel that the relatively strong binding of cysteine to the cysteinyl-tRNA synthetase can be accounted for by a combination of the dispersion energy and hydrogen bonding potential of the thiol group.

Importance of Disperson Energies in Molecular Interactions. Similar reasoning applies to the high bonding energy

of the phenolic hydroxyl of tyrosine with the tyrosyl-tRNA synthetase. In addition to the hydrogen bonding potential of the –OH group, there are the dispersion energy interactions with the protein. Although the dispersion energy is generally ignored, it is seen in Table II that –O– and $-CH_2-$ have similar interaction energies with other $-CH_2-$ groups (the lower polarizability of oxygen is compensated by its shorter van der Waals' radius and, hence, closer contacts). As discussed above, the dispersion energy is expected to be about 2 kcal/mol. Experimental evidence for a significant dispersion energy of the –OH group comes from a comparison of the binding of α-aminobutyrate and threonine to the hydrophobic binding pocket of the valyl-tRNA synthetase (e.g., Fersht & Kaethner, 1976; Fersht & Dingwall, 1979a). Similar values of k_{cat}/K_M (and K_M) for the pyrophosphate exchange reaction show that the *net* binding energy of the hydroxyl group of threonine with the enzyme is about zero. There is thus sufficient dispersion energy with the protein to offset the loss of hydrogen bonding energy of the hydroxyl with water (although there is some gain in entropy on binding threonine, since hydrogen bonded solvent is released).

One approach to analyzing protein–protein interactions is to lump the nonelectrostatic forces into a general "hydrophobic" binding energy, directly proportional to the area of contact between the two protein surfaces, and to assume that the electrostatic forces provide the specificity of binding (Chothia & Janin, 1975). The binding measurements on the aminoacyl-tRNA synthetases, however, indicate that where close packing of surfaces occurs, dispersion energies can provide a significant specificity of binding. Further, they perturb the simple relationship between hydrophobic binding energy and surface area.

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