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Protein Engineering of Tyrosyl-tRNA Synthetase: The Charging of tRNA [and Discussion]

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Protein engineering of tyrosyl-tRNA synthetase: the charging of tRNA

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Protein engineering has been used to identify residues of the tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* that are in contact with the $tRNA^{Tyr}$. By using improved techniques in oligonucleotide-directed mutagenesis, forty lysine, arginine, or histidine residues on the surface of the enzyme were altered to either asparagine or glutamine. With an *in vivo* genetic complementation test, only thirteen mutants were found that seriously affect the overall activity of the enzyme. Detailed kinetics on the purified enzymes revealed that four of these mutants had a lesion at the level of the activation of tyrosine, and nine at the level of tRNA charging. Three of the mutants in tRNA charging lie in the N-terminal domain of the enzyme which is responsible for tyrosine activation, and the six others in the disordered C-terminal domain which is necessary for tRNA binding. This indicates that the tRNA spans both domains of the enzyme. The construction of heterodimers allows us to suggest a model for tRNA binding in which the acceptor stem of the tRNA binds to the N-terminal domain of one subunit, and other regions of $tRNA^{Tyr}$ such as the anticodon arm or extra loop bind to the C-terminal domain of the other subunit.

Introduction

The tyrosyl-tRNA synthetase catalyses the charging of $tRNA^{Tyr}$ with tyrosine, in a two-stage reaction in which the tyrosine is first activated at its carboxyl group with ATP to form tyrosyl adenylate. Subsequently the adenylate is attacked by the 2′ or 3′ hydroxyl of the tRNA to give tyrosyl–$tRNA^{Tyr}$. The three-dimensional structure of the enzyme–tyrosyl adenylate complex, obtained by X-ray crystallography, has allowed the identification of possible hydrogen bonds to the tyrosyl adenylate. Protein engineering experiments, in which the hydrogen-bonding capability of these side chains were removed, have helped to identify their roles in binding to the ground state or transition state (Fersht *et al.* 1984). However, the contacts to the tRNA, or the residues which catalyse the transfer of tyrosine from adenylate to tRNA, have not been identified crystallographically. Both the $tRNA^{Tyr}$ from *Escherichia coli* and that from *B. stearothermophilus* are 85 nucleotides in length, and have a clover-leaf secondary structure with an extra arm consisting of a short base-paired stem and a loop of three nucleotides. Because many of the base pairs stabilizing the tertiary structure of yeast $tRNA^{Phe}$ (Robertus *et al.* 1974) may also be formed in $tRNA^{Tyr}$, the tertiary structures are probably similar. Model-building studies of $tRNA^{Tyr}$, with the use of the known structure of yeast $tRNA^{Phe}$, indicate that the extra loop may lie against the anticodon arm (Brown *et al.* 1978). Thus $tRNA^{Tyr}$ probably folds into an L-shape, with the acceptor stem and TΨC stem forming one arm, and the anticodon stem and extra loop the other arm. The regions of tRNA in contact with the synthetase must include the 2′ or 3′ hydroxyl of the terminal adenosine (Hecht & Craig-Chinault

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1976), and mutants of suppressor $tRNA^{Tyr}$ indicate that the first base pair of the acceptor stem may also contact the synthetase (Celis & Piper 1982). Photo-crosslinking experiments also implicate residue U64 in the T-stem (Ackerman *et al.* 1985) and parts of the helical dihydrouridine arm, the anticodon stem and loop and the extra loop (Schoemaker & Schimmel 1974). Thus the tRNA makes contacts to the synthetase over a wide region, and in particular at both the acceptor stem and anticodon stem and loop (about 7.5 nm apart). We have attempted to identify the contact regions of synthetase by a protein-engineering approach.

A surface-scanning approach

The crystallographic structure of the tyrosyl-tRNA synthetase consists of an ordered N-terminal domain (residues 1–320) which binds tyrosyl adenylate, and a disordered C-terminal domain (residues 321–419) (Bhat *et al.* 1982). The C-terminal domain was excised at the level of the cloned gene by using an M13 splint-deletion technique, and the kinetics of the N-terminal domain assayed. The kinetics of tyrosine activation were identical for the wild-type and for the truncated enzyme, but the truncated enzyme did not charge or bind tRNA. This indicates that the disordered C-terminal domain must contain major binding determinants for the tRNA (Waye *et al.* 1983).

To identify the contacts between the enzyme and tRNA, we used a 'surface-scanning' approach. In such an approach, each surface residue possibly involved in binding tRNA is systematically altered, and the effect on enzyme activity checked. Because the tRNA presents a backbone of negatively charged phosphate groups to solvent, and because tRNA–synthetase binding is dependent on pH (Riesner *et al.* 1976) and ionic strength (Bonnet *et al.* 1975), we suspected ionic interactions. We therefore looked first to the 62 lysine, arginine and histidine residues. A basic side chain is conserved in the *E. coli* tyrosyl–tRNA synthetase at 38 of these sites, and at 10 additional sites, a basic side chain is present in the *E. coli* enzyme within two residues (Winter *et al.* 1983). Because the *E. coli* enzyme can charge *B. stearothermophilus* $tRNA^{Tyr}$ (and the *B. stearothermophilus* enzyme can charge *E. coli* $tRNA^{Tyr}$), the important contacts must be conserved (see Brown *et al.* 1978). We therefore mutated 40 of these residues. Arginine and histidine residues were mutated to glutamine, and lysine to asparagine. This removes the electrostatic charge but retains the hydrophilic nature of the side chain. The construction of the mutants in M13 by using synthetic oligonucleotides was greatly facilitated by the use of repair-deficient cells and strand selection with the use of an amber marker (Carter *et al.* 1985). The mutant lesion on the M13 clone was sequenced, and total sonicate of *E. coli* infected with the mutant M13 phage (heated at 58 °C to eliminate the *E. coli* tyrosyl-tRNA synthetase and most of the *E. coli* proteins) was checked for tyrosyl-tRNA synthetase on SDS polyacrylamide gels (Waye *et al.* 1983). All mutants produced a strong band in the correct position.

Genetic complementation – a quick screen

We suspected that most of the conserved basic residues would not be involved in critical interactions with the tRNA. A quick genetic complementation test was devised to identify mutant enzymes with gross changes in activity. The *B. stearothermophilus tyrS* gene (coding for the tyrosyl-tRNA synthetase) is expressed well in *E. coli* from the M13 clone, and on a plasmid is able to complement a temperature-sensitive lesion in the resident *tyrS* gene of *E. coli* strain

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565CN (Barker 1982). A male *recA* derivative of strain 565CN was constructed, which can now be directly complemented by M13 phage carrying a functional tyrS gene. The complementation test simply involves growing the cells at 30 °C (permissive temperature), and spreading on a plate. The test phage is then spotted onto the plate, and the plate incubated at 42 °C. Colonies will only grow if the phage can complement the ts lesion. Most of the 40 mutants could complement and were therefore eliminated. However, 13 mutants were unable to complement or did so weakly.

Activity of mutants

The 13 mutant enzymes identified from the complementation assay were purified from M13 infected cells and assayed for formation of enzyme-bound tyrosyl adenylate to calculate the number of active sites. The rates of pyrophosphate exchange and tRNA charging were determined (Wilkinson *et al.* 1983). All mutants were able to form tyrosyl adenylate, although for Arg-86→Gln and Lys-233→Asn the rate of formation of adenylate was slow. The pyrophosphate exchange assay revealed that Lys-82→Asn, Arg-86→Gln, Lys-230→Asn and Lys-233→Asn have a lesion at the level of tyrosine activation. In the crystallographic structure, these residues lie on the rim of the active site, and the exact location of the side chains is not clear. Competitive labelling of the synthetase–tRNA complex has indicated that two of these residues, Lys-230 and Lys-233, may be shielded by the tRNA molecule (Bosshard *et al.* 1978). Therefore Lys-230 and Lys-233 may be involved in both tyrosine activation and in tRNA binding.

All the other mutants have a normal rate of exchange but a lesion at the level of tRNA charging. Three of these mutants lie in the ordered N-terminal domain, and the remaining six in the disordered C-terminal domain as a cluster of two and of four (figure 1). This confirms that the C-terminal domain is a major site for tRNA binding. The three mutants in the ordered N-terminal domain map along a line leading away from the active site. Detailed kinetics demonstrate that Lys-151→Asn has an unchanged K_m (tRNA) for tRNA charging, but a

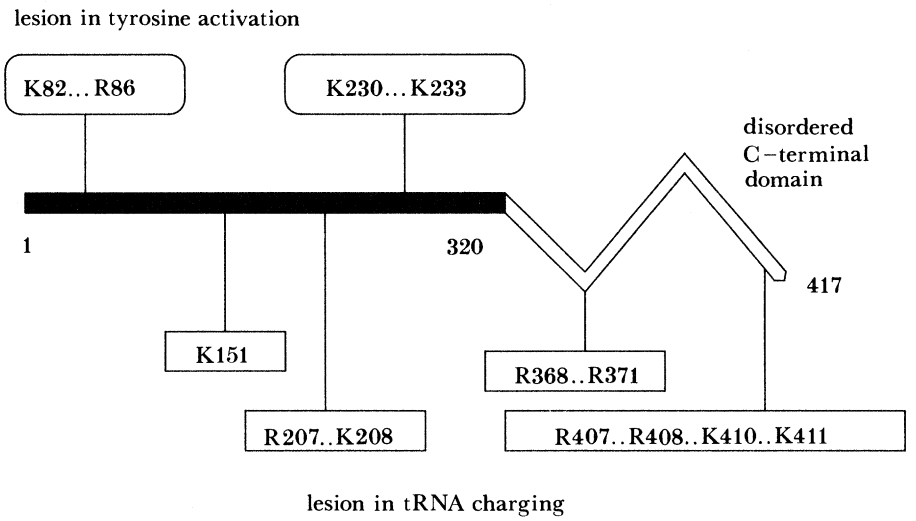

Figure 1. Locations of mutants detected by 'surface scanning'.

dramatically reduced k_{cat}, while Arg-207→Gln and Lys-208→Asn have a dramatically increased K_m. Lys-151 is closer to the active site, and the fact that the lesion mainly affects the rate may indicate that this contact helps orientate the 3′ end of the tRNA for the attack on the adenylate. Presumably Arg-207 and Lys-208 anchor the acceptor stem of the tRNA. The mutants in the C-terminal disordered domain all have dramatically increased K_m values. By comparing the k_{cat}/K_m (tRNA) values for mutant and wild-type enzymes, we find that the loss in interaction energy at each contact ranges between 7.1 and 12.1 kJ mol^{-1}.

Construction of heterodimers

The tRNA molecule clearly makes contacts with both the N-terminal domain and the disordered C-terminal domain, and the tRNA must therefore span both domains. Because the enzyme is a dimer, the tRNA must bind to the N-terminal domain of one subunit and either the C-terminal domain of the same subunit or the C-terminal domain of the other subunit. To distinguish between these possibilities, we constructed heterodimers between the truncated enzyme (which lacks the entire C-terminal domain) and a mutant (His-45→Asn) which has a lesion at the active site. The His-45→Asn mutant forms tyrosyl adenylate but with a k_{cat} reduced by 3000-fold, and is therefore almost inactive in tRNA charging (Fersht *et al.* 1984). Thus one of the parent homodimers is active in tyrosine activation, and both are inactive in tRNA charging. However, the mutant heterodimer, constructed by first denaturing and folding the two parent homodimers together in 8 M urea and then refolding and purifying on a native polyacrylamide gel, proved to be active in both tyrosine activation and in tRNA charging (table 1).

Table 1. Kinetic parameters for the heterodimer constructions

	pyrophosphate exchange		tRNA charging	
dimer	K_m(ATP)/mM	k_{cat}/s^{-1}	K_m(ATP)/mM	k_{cat}/s^{-1}
WT/WT	1.4	6.25	0.56	2.25
His-45→Asn/WTt	1.2	3.44	0.52	1.26
WT/His-45→Asnt	1.2	3.25	—	—

The heterodimers were prepared by mixing the parent homodimers in 10 M urea and electrophoresing into a 10% native polyacrylamide gel. Because of their different sizes, the parent homodimers and the heterodimers are readily separated, and were located on the gel by staining a diffusion blot of the gel on nitrocellulose with Indian ink. The bands were cut from the gel with a razor, and the heterodimers recovered by electro-elution, concentrated on DEAE–cellulose and finally dialysed against kinetic assay buffer. The heterodimers were checked on an SDS polyacrylamide gel to prove that the two subunits were of different size. Active-site titration, pyrophosphate exchange and tRNA charging assays were as described previously (Wilkinson *et al.* 1983). WT, WTt and His-45→Asnt refer to the wild-type, truncated and mutant truncated enzymes respectively.

In the activation step, the refolded heterodimer has K_m values for tyrosine and ATP that are almost identical to the wild-type enzyme, and the number of active sites (bound tyrosyl adenylate) per mole of heterodimer is identical to that of the parent homodimers (one active site per mole dimer). However, the k_{cat} value for the heterodimer is one-half of that of wild-type enzyme: this is probably a consequence of the enzyme's 'half of sites reactivity', in which substrate binding to one subunit switches off the other subunit. Thus binding of tyrosine to the subunit with the His-45→Asn lesion switches off the active subunit in the heterodimer,

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while binding to the active subunit yields tyrosyl adenylate, resulting overall in half the k_{cat} per active site.

In tRNA charging, the K_m (ATP) is unaffected in the heterodimer, but the k_{cat} is one-half that of wild-type enzyme. Thus the heterodimer appears to be a fully refolded, active molecule, in which one subunit can complement a lesion on the other subunit. This suggests two alternatives: either both subunits are involved in tRNA binding (figure 2), or tyrosyl adenylate

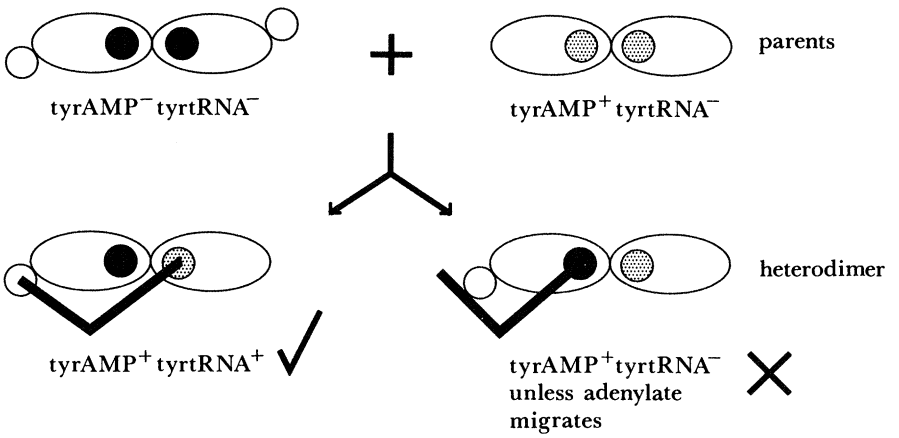

FIGURE 2. A model of tRNA interaction with the synthetase as elucidated by heterodimer construction.

can migrate from one active site to the other. However, the heterodimer constructed from wild-type dimer and truncated enzyme with a His-45→Asn lesion was inactive, ruling out migration of adenylate (table 1).

Similar heterodimer constructions were made between each of the nine mutants in the tRNA charging step, and the truncated enzyme. Thus each of the parents, while active in tyrosine activation, are inactive in tRNA charging. Most of the heterodimers were inactive in tRNA charging: however, those having a mutation in the N-terminal domain (Lys-151→Asn, Arg-207→Gln and Lys-208→Asn) proved to be active as expected if both subunits are involved in tRNA binding. Likewise a heterodimer constructed between Lys-151→Asn and Arg-368→Gln (both parents inactive in tRNA charging) proved to be active.

MODEL OF INTERACTION OF tRNA AND SYNTHETASE

The 3′ end of the tRNA must attack the tyrosyl adenylate at the active site, and it seems likely that the acceptor stem interacts with Lys-151, Arg-207 and Lys-208. Other contacts to the tRNA, perhaps the extra arm and the anticodon arm, are made from two basic clusters in the disordered C-terminal domain of the other subunit. We have accordingly attempted a docking of the synthetase (Bhat *et al.* 1982) with $tRNA^{Phe}$ (Robertus *et al.* 1974), by using the computer graphics programme FRODO. The 3′ hydroxyl of yeast $tRNA^{Phe}$ was held near the ester linkage of tyrosyl adenylate in the active site, and the tRNA rotated so that the phosphodiester backbone of the acceptor stem came into the proximity of Lys-151, Arg-207 and Lys-208. In the resulting model, the -CCA end and possibly as much as the first half-turn

of the acceptor stem could bind to the N-terminal domain. However, the model can only be crude, as the $tRNA^{Tyr}$ structure is unknown and the terminal -CCA is flexible.

With the path of the acceptor arm fixed as above, we have examined the alternatives for interaction of the anticodon arm or extra loop with the other subunit. We find that it is possible to accommodate the binding of the tRNA across both subunits as an 'L' shape, without a transition to a 'U' shape (Reid 1977). By rotating the tRNA about the axis which runs down the acceptor stem, it is possible to make three types of interactions with the other subunit. At one extreme, the tRNA could straddle the two subunits on only one face of the dimer, interacting with the C-terminal island of density (Irwin *et al.* 1976; Bhat *et al.* 1982). Sweeping the anticodon arm round further, it can interact with the rim of the second active site. This might explain the protection of Lys-230 and Lys-233 by tRNA from attack by acetic anhydride (Bosshard *et al.* 1978). However, heterodimers constructed between the truncated synthetase and the mutants at 82, 86, 230 and 233 are active in tRNA charging, implying that they do not make essential contacts to the tRNA from the second subunit.

At the other extreme, the arm pit of the tRNA could fit in the groove between the two subunits of the synthetase. Such a mode of interaction was proposed in the 'symmetry recognition' model, in which the pseudo-symmetric regions of a tRNA are recognized by symmetric regions on the synthetase (Kim 1975). One prediction of symmetry recognition is that the same amino-acid residue will interact twice with the tRNA, once from one subunit and once from the other subunit. The fact that we can make heterodimers active in tRNA charging with Lys-151, Arg-207 and Lys-208 mutants and the truncated enzyme, implies that these three residues in the N-terminal domain and the entire C-terminal domain make single contacts with the tRNA. These residues at least are not therefore involved in symmetry recognition. However, it is still possible that the tRNA might pass between the two subunits, and it would then pass close to Lys-210, and to Lys-140 and His-141, which line the inside of the groove. Mutation of these side chains yields active enzyme, implying that they do not make essential contacts to the tRNA.

Thus in the model we prefer, the tRNA docks to the synthetase dimer via the acceptor stem on one subunit, and via the anticodon arm to the disordered C-terminal domain of the other subunit such that the tRNA lies across one face of the dimer. Further experiments to test the salient features of this model might include mutagenesis of surface residues in the vicinity of Lys-151, Arg-207 and Lys-208 to delineate the contacts to the acceptor stem. Comparing the protection of the tRNA from chemical or nuclease attack by wild-type and mutant enzyme, might show whether the anticodon arm (and extra arm) are indeed in contact with the C-terminal domain as predicted.

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References

Ackerman, E. J., Joachimiak, A., Klinghofer, V. & Sigler, P. B. 1985 *J. molec. Biol.* **181**, 93–102.
Barker, D. G. 1982 *Eur. J. Biochem.* **125**, 357–360.
Bhat, T. N., Blow, D. M., Brick, P. & Nyborg, J. 1982 *J. molec. Biol.* **158**, 699–709.
Bonnet, J., Renaud, M., Raffin, J. P. & Remy, P. 1975 *FEBS Lett.* **53**, 154–158.
Bosshard, H. R., Koch, G. L. E. & Hartley, B. S. 1978 *J. molec. Biol.* **119**, 377–389.
Brown, R. S., Rubin, J. R., Rhodes, D., Guilley, H., Simoncsits, A. & Brownlee, G. G. 1978 *Nucl. Acids Res.* **5**, 23–36.
Carter, P., Bedouelle, H. & Winter, G. 1985 *Nucl. Acids Res.* **13**, 4431–4443.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Celis, J. E. & Piper, P. W. 1982 *Nucl. Acids Res.* **10**, r83–r91.
Fersht, A. R., Shi, J.-P., Wilkinson, A. J., Blow, D. M., Carter, P., Waye, M. M. Y. & Winter, G. P. 1984 *Angew. Chem.* **23**, 467–473.
Hecht, S. M. & Craig-Chinault, A. 1976 *Proc. natn. Acad. Sci. U.S.A.* **73**, 405–409.
Irwin, M. J., Nyborg, J., Reid, B. R. & Blow, D. M. 1976 *J. molec. Biol.* **105**, 577–586.
Kim, S. H. 1975 *Nature, Lond.* **256**, 679–681.
Reid, B. R. 1977 In *Nucleic acid protein recognition* (ed. H. J. Vogel), pp. 375–390. New York: Academic Press.
Riesner, D., Pingoud, A., Boehme, D., Peters, F. & Maass, G. 1976 *Eur. J. Biochem.* **68**, 71–80.
Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C. & Klug, A. 1974 *Nature, Lond.* **250**, 546–551.
Schoemaker, H. J. P. & Schimmel, P. R. 1974 *J. molec. Biol.* **84**, 503–513.
Waye, M. M. Y., Winter, G., Wilkinson, A. J. & Fersht, A. R. 1983 *EMBO J.* **2**, 1827–1829.
Wilkinson, A. J., Fersht, A. R., Blow, D. M. & Winter, G. 1983 *Biochemistry* **22**, 3581–3586.
Winter, G., Koch, G. L. E., Hartley, B. S. & Barker, D. G. 1983 *Eur. J. Biochem.* **132**, 383–387.

Discussion

P. Brick (*Biophysics Group, Imperial College, London*). Dr Winter has explained how the choice of mutation sites has been based on our knowledge of the three-dimensional crystal structure of the enzyme (Bhat *et al.* 1982; Blow & Brick 1985). To analyse the strength and nature of enzyme–substrate interactions, and to correlate them with detailed kinetic measurements, it is essential to obtain an accurate molecular model at the highest possible resolution.

The major problem that hampered us in determining an accurate structure for the enzyme was that about 100 amino acids at the C-terminus of the molecule were disordered in the wild-type crystals, and so we had been unable to build a molecular model for this part of the molecule. This resulted in less accurate phases, which introduced uncertainties in the remaining three-quarters of the structure. We were able to circumvent these problems to some extent by calculating structure factors for the uninterpreted electron density and by adding these to those calculated from the atomic model (Bhat & Blow 1982). Even so the final molecular model was still not as satisfactory as that obtainable from better ordered crystals.

Mary Waye and her co-workers (Waye *et al.* 1983) removed the DNA coding for the disordered C-terminal region from the structural gene and expressed this deletion mutant in *Escherichia coli*. They showed that the truncated enzyme catalysed the formation of tyrosyl adenylate but no longer bound $tRNA^{Tyr}$ or transferred tyrosine to $tRNA^{Tyr}$. They were able to obtain good yields of pure enzyme and we hoped that by solving the structure of this mutant we would obtain a better molecular model.

Crystals were obtained from the truncated enzyme at neutral pH in the presence of tyrosine by using polyethylene glycol as a precipitant. The space group is $P2_1$, with cell dimensions $a = 94.5$ Å†, $b = 67.1$ Å, $c = 61.4$ Å and $\beta = 90.78°$. The crystallographic asymmetric unit contains both subunits of the dimeric molecule. A three-dimensional data set was collected to 2.5 Å resolution by using an Arndt–Wonacott oscillation camera.

The position of the truncated molecule in the crystallographic asymmetric unit of this new crystal form was determined by using standard molecular replacement techniques. The search object comprised the 270 better ordered residues from the refined native structure. Restrained least-squares structure-factor refinement, with the use of the computer program PROLSQ (Konnert & Hendrickson 1980) with alternating cycles of model building lowered the *R*-factor to the current value of 21 %.

† 1 Å = 10^{-1} nm = 10^{-10} m.

This new molecular model has greatly improved stereochemistry and includes about 50 water molecules in each subunit. Most of these are situated in and around the active-site cleft. Residues that had higher atomic temperature factors in the previous model generally retain high temperature factors in the current model, but there are now clear electron density data for many of these less well ordered residues, providing a more reliable molecular model.

The substrate tyrosine and the surrounding protein side chains are particularly well ordered, allowing us to be confident about their interactions. The phenolic ring of the tyrosine substrate sits at one end of a narrow slot at the bottom of the active site cleft (figure D1). The other end of this slot is occupied by a string of well ordered water molecules. The hydroxyl and α-amino groups of the substrate are in a position to make multiple hydrogen bonds with protein side chains. In contrast, the carboxyl group appears to form no hydrogen bonds with the enzyme. The side chains that form this binding slot are involved in an extremely complex network of hydrogen bonds (figure D2).

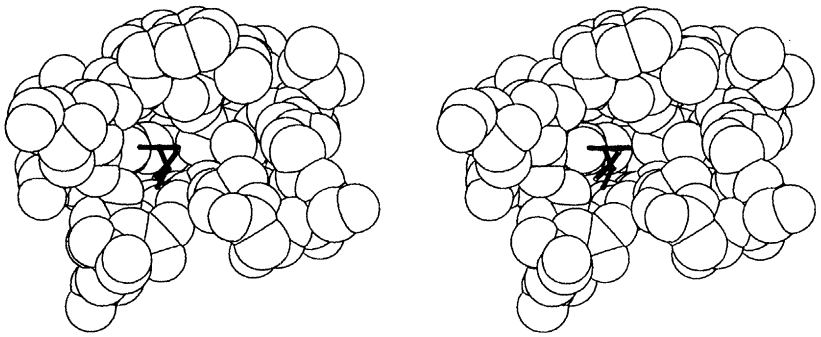

FIGURE D1. Van der Waals drawing showing the tyrosine binding pocket. The substrate tyrosine is represented as a stick model. The substrate lies in the left side of the slot, while three well ordered water molecules are found in the space to the right of it.

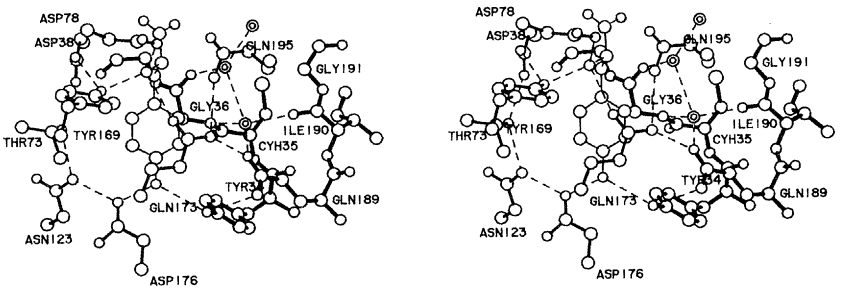

FIGURE D2. Some of the amino-acid side chains composing the tyrosine binding pocket. The tyrosine substrate is shown with lighter bonds. Hydrogen bonds are shown as broken lines. The well ordered water molecules observed in the substrate binding slot are indicated by double circles.

It has already been demonstrated that by cloning a fragment of a protein one can produce a molecule more amenable to crystallization and structure determination (Joyce & Grindley 1983; Ollis *et al.* 1985). This technique is particularly suitable where the fragment retains enzymatic activities of the parent molecule. It has been used here to improve the structural information obtained from crystals of the intact molecule. The improved model will be particularly valuable in the analysis of other mutants of tyrosyl tRNA synthetase.

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References

Bhat, T. N., Blow, D. M., Brick, P. & Nyborg, J. 1982 Tyrosyl-tRNA synthetase forms a mononucleotide-binding fold. *J. molec. Biol.* **158**, 699–709.

Bhat, T. N. & Blow, D. M. 1983 A method for refinement of partially interpreted protein structures including a procedure for scaling between a model and an electron-density map. *Acta crystallogr.* A **39**, 166–170.

Blow, D. M. & Brick, P. 1985 Aminoacyl-tRNA Synthetases. In *Biological macromolecules and assemblies*, vol. 2 (ed. F. A. Jurnak & A. McPherson), pp. 442–469. New York: Wiley.

Joyce, C. M. & Grindley, N. D. F. 1983 Construction of a plasmid that overproduces the Klenow fragment of DNA polymerase I of *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1830–1834.

Ollis, D. L., Brick, P., Hamlin, R., Xuong, N. G. & Steitz, T. A. 1985 Structure of large fragment of *Escherichia coli* DNA polymerase I complexed with dTMP. *Nature, Lond.* **313**, 762–766.

Waye, M. M. Y., Winter, G., Wilkinson, A. J. & Fersht, A. R. 1983 Deletion mutagenesis using an 'M13 Splint'. *EMBO J.* **2**, 1827–1829.