Conserved cysteine and histidine residues in the structures of the tyrosyl and methionyl-tRNA synthetases

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Aminoacyl-tRNA synthetase *Protein structure homology*

1. INTRODUCTION

Aminoacyl-tRNA synthetases catalyse the aminoacylation of tRNA molecules with their respective amino acids (review, see [1]). In most cases, including the bacterial tyrosyl-tRNA synthetase (TyrTS) and methionyl-tRNA synthetase (MetTS), the amino acid is first activated with ATP to the aminoacyl adenylate, and is then transferred to its cognate tRNA [2–4]. The structural constraints on the binding sites for the three substrates (amino acid, ATP and tRNA) might be expected to impose extensive common structural features on the synthetases, especially as all tRNAs probably share a common tertiary conformation [5] and interact with the synthetase over a large surface area [6].

Until recently there has been little experimental evidence to support such expectations. On the contrary, the quaternary structures of these enzymes are extremely diverse, ranging from the dimeric 2 × 37 000 dalton tryptophanyl-tRNA synthetase (TrpTS) to the tetrameric 4 × 95 000 dalton alanyl-tRNA synthetase (AlaTS) [7,8]. Similarly, searches for primary sequence homology between TrpTS (*B. stearothermophilus*) and TyrTS (*B. stearothermophilus*) [9] or between AlaTS (*E. coli*) and TyrTS (*E. coli*) [10] have proved inconclusive. However, high resolution electron density maps have now revealed that the structures of TyrTS from *B. stearothermophilus* [11] and a proteolytic fragment of MetTS from *E. coli* [12] bear certain topological similarities, in particular an alternating α/β N-terminal domain with a Rossman fold of β-sheet [13]. In the TyrTS structure, the activated amino acid (tyrosyl adenylate) binds to a specific site at the C-terminal end of the Rossman fold, thus identifying this region as part of the enzyme's active site [14].

Via DNA sequence we have determined the primary sequences of the MetTS [15] and TyrTS [16] and have identified a short stretch of homology between the two sequences, involving a constellation of a cysteine and two histidine residues. These residues occupy homologous positions in the three-dimensional structures, lying close to the active site, and we propose that they may be directly involved in the catalytic mechanism of amino acid activation and its transfer to cognate tRNA.

2. METHODS

A computer program (DIAGON) was kindly provided by R. Staden [17] in which all sections of the TyrTS (*B. stearothermophilus*) and MetTS (*E. coli*) were compared in a manner similar to McLachlan [18]. These analyses were displayed on a graphics terminal, any matches appearing as diagonal lines.

3. RESULTS AND DISCUSSION

The computer search for homology located a match of seven amino acids between the two enzymes (fig.1). There are two histidine residues within this short homologous sequence, and in addition we find a cysteine residue at a distance of exactly 10 amino acids before the first of these histidines. The presence of possible cysteine and histidine homologies is interesting because both imidazole and thiol groups have been implicated in

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                    5                  10                  15                  20                  25                  30
MetTS (E.coli)      GlnValAlaLysLysIleLeuValThr Cys AlaLeuProTyrAlaAsnGly SerIleHisLeuGlyHisMet LeuGluHisIleGlnAla  [15]
                              30                  35                  40                  45                  50                  55
TyrTS (B.stearo.)   LeuAsnGluGluArgValThrLeuTyr Cys GlyPheAspProThrAlaAsp SerLeuHisIleGlyHisLeu AlaThrIleLeuThrMet  [16]

                   170                 175                 180                 185                 190                 195
AlaTS (E.coli)      PheTrpArgMetGlyGlyThrGlyPro Cys AspProCysThrGluIlePhe TyrAspHisGlyAspHisIle TrpGlyGlyProProGly  [10]
                              30                  35                  40                  45                  50                  55
TrpTS (B.stearo.)   PheValGluLeuGlnHisGlxTyrAsn Cys TyrPheCysIleValAsxGlx HisAlaIleThrValTrpGln AspProHisGluLeuArg  [19]
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Fig.1. Alignment of the relevant amino acid sequences of *E. coli* MetTS and *B. stearothermophilus* TyrTS in order to show the seven amino acid homologous regions and the conserved cysteine residues (boxed). Short stretches of the *E. coli* AlaTS and *B. stearothermophilus* TrpTS sequences are also included for comparative purposes, as discussed in the text. The numbering above each sequence indicates the distance from the N-terminus.

the catalytic mechanism of aminoacyl-tRNA synthetases (see below). The identification of Cys^{35} of TyrTS (*B. stearothermophilus*) in this constellation is particularly satisfying as this is the only cysteine residue which it has in common with TyrTS of *E. coli* [16].

Although the alignment of Ser^{19}–Met^{25} (MetTS) with Ser^{43}–Leu^{49} (TyrTS) seems very clear in the comparison of the two sequences, the double matching probability [18] is only about 10^{-5} and could easily arise by chance. However, a subsequent detailed inspection of the crystal structures of MetTS (by courtesy of Drs J.L. Risler and C. Zelwer, and of TyrTS, by courtesy of Prof. D.M. Blow), has revealed that the constellation of histidine and cysteine residues occupy homologous positions in the two structures. Residues 31–61 of the TyrTS form part of the Rossman fold structure, contributing the first central parallel β-strand and one of the α-helices (shown schematically in fig.2). Cys^{35} lies near the middle of the β-sheet and His^{45} and His^{48} are located near the top of the α-helix, with Pro^{39} marking the turn at the top of the β-strand. Thus the two histidine residues lie close to the C-terminal end of the β-sheet and hence to the tyrosyl adenylate binding site. In the MetTS structure the residues Cys^{11}, Pro^{14}, His^{21} and His^{24} all occupy homologous positions.

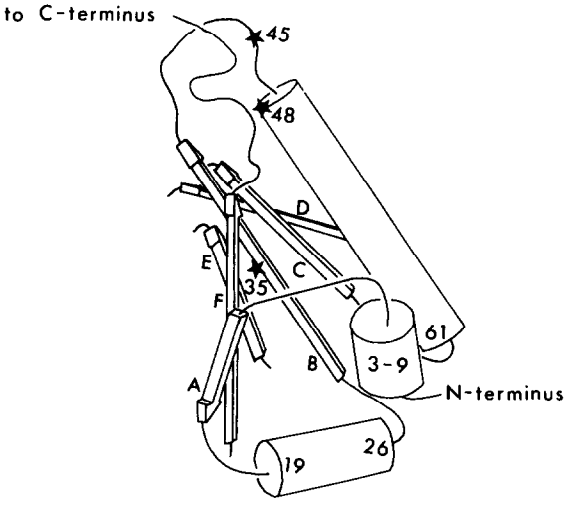

Fig.2. Schematic representation of part of the Rossman fold of the *B. stearothermophilus* TyrTS (reproduced by kind permission of Prof. D.M. Blow) showing the single anti-parallel (A) and five parallel (B–F) β-strands and the first three connecting α-helices. The amino acid numbering is the same as in fig.1, and the asterisks mark the approximate locations of the cysteine and histidine residues.

The discovery of this conserved sequence led us to search for a similar pattern in the amino acid sequences of the TrpTS (*B. stearothermophilus* [19] and AlaTS (*E. coli*) [10]. In TrpTS a stretch of sequence between residues 31–52 contains both cysteine and histidine residues but their relative positions cannot be directly correlated with the motif in TyrTS and MetTS. We must therefore await a comparison of tertiary structures [20]. In the AlaTS, the spacing of Cys^{178}, His^{188} and His^{191} does conform to the motif but the surrounding amino acids are not homologous to either the TyrTS or MetTS sequences (fig.1). Thus the TyrTS and MetTS, in containing a snippet of conserved primary sequence at the active site, may be more closely related to each other in evolution than to either the AlaTS or TrpTS.

Is there any evidence that the histidine and cysteine residues are directly involved in catalysis in TyrTS or MetTS? The modification of (a) cysteine

residue(s) in MetTS can prevent formation of the methionyl adenylate and furthermore the aminoalkyl analogue (methioninol adenylate) is capable of inhibiting this modification [21]. Also in MetTS, as in several other synthetases [21–28], the modification of thiol groups results in a complete loss of tRNA acylation activity and it has been proposed that a thioacyl intermediate is involved in the transfer of amino acid to tRNA [29]. Histidine residues have also been proposed as covalent intermediates in both catalytic steps [30] and implicated experimentally in the enzymatic activities of the TrpTS (beef pancreas) [31] and the PheTS (*E.coli* and yeast) [32,33]. Although comparable experiments have not been described for TyrTS or MetTS we could expect that the chemical modification of the conserved histidines (or cysteine) would destroy enzyme activity if only because these residues are located near the active site. The answer as to whether these residues actually form part of the catalytic mechanism or are involved in making critical contacts is at present equivocal, but their chemical repertoire [29,30], location at the active site and conservation in two widely different sequences strongly suggests that they do.

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REFERENCES

[1] Schimmel, P.R. and Söll, D. (1979) Ann. Rev. Biochem. 48, 601–648.
[2] Fersht, A.R. and Jakes, R. (1975) Biochemistry 14, 3350–3356.
[3] Blanquet, S., Fayat, G. and Waller, J.-P. (1974) Eur. J. Biochem. 44, 343–351.
[4] Loftfield, R.B. (1972) Prog. Nucl. Acids Res. 12, 87–128.
[5] Moras, D., Comarmond, M.B., Fischer, J., Weiss, R., Thierry, J.C., Ebel, J.P. and Giegé, R. (1980) Nature 288, 669–674.
[6] Rich A. and Schimmel, P.R. (1977) Nucleic Acids Res. 4, 1649–1665.
[7] Koch, G.L.E., Boulanger, Y. and Hartley, B.S. (1974) Nature 249, 316–320.
[8] Putney, S.D., Sauer, R.T. and Schimmel, P.R. (1981) J. Biol. Chem. 256, 198–204.
[9] Winter, G.P., Koch, G.L.E., Dell, A. and Hartley, B.S. (1979) in: Transfer RNA: Structure Properties and Recognition (Schimmel, P.R., Söll, D. and Abelson, J.N. eds). pp. 255–265. Cold Spring Harbor Laboratory, New York.
[10] Putney, S.D., Royal, N.J., De Vegvar, H.N., Herlihy, W.C., Biemann, K. and Schimmel, P.R. (1981) Science, 213 1497–1500.
[11] Bhat, T.N., Blow, D.M., Brick, P. and Nyborg, J. (1982) J. Mol. Biol., in press.
[12] Zelwer, C., Risler, J.L. and Brunie, S. (1982) J. Mol. Biol. 155, 63–81.
[13] Risler, J.L., Zelwer, C. and Brunie, S. (1981) Nature 292, 384–386.
[14] Rubin, J. and Blow, D.M. (1981) J. Mol. Biol. 145, 489–500.
[15] Barker, D.G., Ebel, J.P., Jakes, R. and Bruton, C.J., (1982) submitted.
[16] Barker, D.G., Hartley, B.S., Koch, G.L.E. and Winter, G.P., (1982) in preparation.
[17] Staden, R. (1982) Nucleic Acids Res., in press.
[18] McLachlan, A.D. (1971) J. Mol. Biol. 61, 409–424.
[19] Winter, G.P. and Hartley, B.S. (1977) FEBS Lett. 80, 340–342.
[20] Carter, C.W. and Carter, C.W. (1979) J. Biol. Chem. 254, 12219–12223.
[21] Cassio, D. (1968) Eur. J. Biochem. 4, 222–224.
[22] Stern, R., DeLuca, M., Mehler, A.M. and McElroy, W.D. (1966) Biochemistry 5, 126–130.
[23] Kuo, T. and DeLuca, M. (1969) Biochemistry 8, 4762–4768.
[24] Rouget, P. and Chapeville, F. (1971) Eur. J. Biochem. 23, 452–458.
[25] Ostrem, D.L. and Berg, P. (1974) Biochemistry 13, 1338–1348.
[26] Murayama, A., Raffin, J.P., Remy, P. and Ebel, J.P. (1975) FEBS Lett. 53, 15–22.
[27] Rainey, P., Holler, H. and Kula, M.R. (1976) Eur. J. Biochem. 63, 419–426.
[28] Renaud, M., Fasiolo, F., Baltzinger, M., Boulanger, Y. and Remy, P. (1982) Eur. J. Biochem. 123, 267–274.
[29] McElroy, W.D., DeLuca, M. and Travis, J. (1967) Science 157, 151–160.
[30] Krayevsky, A.A., Kisselev, L.L. and Gottikh, B.P. (1973) Molek. Biol. 7, 769–775.
[31] Favorova, O., Madoyan, I.A. and Kisselev, L.L. (1978) Eur. J. Biochem. 86, 193–202.
[32] Hennecke, H. and Böck, A. (1974) Eur. J. Biochem. 50, 157–166.
[33] Raffin, J.P. and Remy, P. (1978) Biochim. Biophys. Acta 520, 164–174.