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Origin of Glutaminyl-tRNA Synthetase: An Example of Palimpsest?

Massimo Di Giulio

International Institute of Genetics and Biophysics, CNR, Via G. Marconi 10, 80125 Naples, Napoli, Italy

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Abstract. Sequence data and evolutionary arguments suggest that a similarity may exist between the C-terminal end of glutaminyl-tRNA synthetase (GlnRS) and the catalytic domain of glutamine amidotransferases (GATs). If true, this would seem to imply that the amidation reaction of the Glu-tRNA^{Gln} complex was the evolutionary precursor of the direct tRNA^{Gln} aminoacylation pathway. Since the C-terminal end of GlnRS does not now have an important functional role, it can be concluded that this sequence contains vestiges that lead us to believe that it represents a palimpsest. This sequence still conserves the remains of the evolutionary transition: amidation reaction → aminoacylation reaction. This may be important in deciding which mechanism gave origin to the genetic code organization. These observations, together with results obtained by Gatti and Tzagoloff [J. Mol. Biol. (1991) 218: 557–568], lead to the hypothesis that the class I aminoacyl-tRNA synthetases (ARSs) may be homologous to the GATs of the *trpG* subfamily, while the class II ARSs may be homologous to the GATs of the *purF* subfamily. Overall, this seems to point to the existence of an intimate evolutionary link between the proteins involved in the primitive metabolism and aminoacyl-tRNA synthetases.

Key words: Palimpsest proteins — Mosaic proteins — Recruitment of antique catalytic domains — Homology — Glutamine amidotransferases — CTP synthetase — Origin of aminoacyl tRNA synthetases — Genetic code — Origin of life

Introduction and Hypothesis

There is considerable data favoring the hypothesis of a coevolution of the biosynthetic relationships between amino acids and the organization of the genetic code (Wong 1975, 1976, 1980; Wong and Bronskill 1979; Levy and Danchin 1988; Miseta 1989; Di Giulio 1992). Wong (1975) proposed that the transformations of the precursor amino acid into the product, taking place according to his hypothesis on the tRNA-like molecule, were mainly responsible for the mechanism that gave rise to the organization of the genetic code. One of the clearest indications that these homeotopic transformations (Danchin 1989) may have been involved in the structuring of the genetic code derives from the amidation of glutamic acid while this is charged on a tRNA specific for glutamine (Wilcox and Nirenberg 1968; Schon et al. 1988). This pathway for the formation of the Gln-tRNA^{Gln} complex is found in archaebacteria (Gupta 1984), cyanobacteria, Gram-positive eubacteria, and in chloroplasts and mitochondria (Schon et al. 1988). In these cases the presence of glutaminyl-tRNA synthetase (GlnRS) was not detected and it was, rather, glutamyl-tRNA synthetase that charged glutamic acid on the tRNA^{Gln} (Schon et al. 1988). In the cytoplasm of eukaryotes there is no evidence of the existence of the Glu-tRNA^{Gln} → Gln-tRNA^{Gln} pathway and the presence of GlnRS is detected. The distribution of the Glu-tRNA^{Gln} → Gln-tRNA^{Gln} pathway in the three main lines of divergence leads us to believe that this characteristic may have belonged to the

progenote. If this pathway is an acquired, and not a primitive trait, i.e., if it is due to a convergence phenomenon in eubacteria and archaebacteria, it is difficult to see which selective pressure favored the development of this pathway, as the mischarging of the $tRNA^{Gln}$ with Glu would, in any case, have compromised the accuracy of the translation of the genetic message, and this would have been selected against. On the other hand, GlnRS is present, at least, in both eukaryotes and eubacteria. Here too it is more parsimonious to hypothesize that GlnRS was a trait possessed by the last common ancestor between these phylogenetic lines, and if the GlnRSs of the eubacteria and eukaryotes are homologous, as seems to be the case (Nagel and Doolittle 1991), then the line of divergence that led, for instance, to archaebacteria, on the basis of the small amount of data available (Gupta 1984), may have lost GlnRS or may never have evolved it and, rather, may have maintained the presumed primitive pathway Glu-$tRNA^{Gln} \rightarrow$ Gln-$tRNA^{Gln}$.

The observation that the allocations of amino acids in the genetic code are related to the precursor-product amino acid transformations (Wong 1975), together with the data indicating a considerable heterogeneity both in the primary and quaternary structures of the aminoacyl-tRNA synthetases (ARSs) specific for different amino acids (Schimmel 1987), led to the formulation of the following working hypothesis. It can be hypothesized that the ARSs evolved from (have a common origin with) one of the enzymes that catalyzed one of the reactions that now led to the biosynthesis of the respective amino acid. If the precursor-product amino acid transformations occurred on a tRNA-like molecule (Wong 1975), the ancestral peptide favoring this transformation would have had a certain specificity both for the precursor-tRNA complex (or for the intermediate metabolite-tRNA) and at least partly for the product-tRNA and, thus, would have been equivalent to an ARS. This working hypothesis would therefore explain the observed heterogeneity between ARSs (Schimmel 1987) by means of the observation that the biosynthetic relationships between amino acids are reflected in the organization of the genetic code (Wong 1975).

The mechanism by which the Glu-$tRNA^{Gln}$ complex is transformed into Gln-$tRNA^{Gln}$ in the organisms or organelles without GlnRS (Schon et al. 1988; Jahn et al. 1990) seems to provide a first proof in favor of our working hypothesis. As suggested by Schon et al. (1988), the amidotransferase that transforms the Glu-$tRNA^{Gln}$ complex into Gln-$tRNA^{Gln}$ seems to be formally similar to GlnRS: both enzymes possess specific bonding sites for glutamine, ATP, and $tRNA^{Gln}$. Furthermore, this example of homeotopic modification (Danchin 1989) seems to make the working hypothesis more particularized. This example seems to suggest that there must be a similarity in the primary structure between GlnRS and a glutamine-dependent amidotransferase: hence, evidence of the existence of homology.

I have, therefore, searched for evidence favoring similarity in the amino acid sequence between GlnRS and glutamine amidotransferases.

Materials and Methods

The glutaminyl-tRNA synthetase (GlnRS) sequence was taken from Hoben et al. (1982: Fig. 2), while the CTP synthetase (CTPS) sequence was taken from Weng et al. (1986: Fig. 3). Both sequences are from *E. coli*. The sequence of the GlnRS of *S. cerevisiae* was taken from Ludmerer and Schimmel (1987: Fig. 2). In this paper reference will be made to Fig. 7 in Weng et al. (1986), which shows the three conserved segments of the catalytic domain of glutamine amidotransferases (GATs) of type *trpG*.

The comparison between sequences was conducted using the programs contained in FASTA (Pearson and Lipman 1988). Specifically, the following programs were used: (1) The LFASTA program (1.4c2; June 1990), which detects local similarities between two sequences, was used at the beginning of research. This program identified a region of similarity between CTPS and GlnRS using the first region shown in Fig. 5B of Yamauchi et al. (1990) as a segment of CTPS (i.e., the one containing two of the conserved segments of GATs) which was compared with the whole GlnRS sequence. In this way it was possible to identify a region of similarity of 25 amino acids (the first 25 amino acids in Fig. 1) with an identity percentage of 40%. (2) The program ALIGN (1.0; April 1988) optimizes the alignment between the two sequences. (3) The program RDF2 (August 1988) determines the statistical significance of a specific alignment between two sequences. This program was used in most of the analyses by setting the following parameter values: ktup = 1, number of random shuffles = 100, and uniform shuffle = u. In all the cases reported here, the two sequences were used as both a test sequence and a shuffled sequence. The values of the standard deviations above the mean of the optimized scores and the other scores are the mean of at least 10 trials.

Results

The amide group of glutamine is a source of nitrogen in the biosynthesis of a variety of compounds. These reactions are catalyzed by a group of enzymes known as glutamine amidotransferases (GATs) (Kaplan et al. 1985). The comparison of the amino acid sequences of these enzymes has shown that there are two distinct subfamilies of GATs, designated as *trpG* and *purF* (Weng and Zalkin 1987; Mei and Zalkin 1989).

If at least one region of the glutaminyl-tRNA synthetase (GlnRS) is actually homologous to the catalytic domain of GATs, then a region of the synthetase might show traces of sequence similarity with this domain. By using the programs contained in FASTA (see Materials and Methods) I have found that the C-terminal end of the GlnRS of *E. coli*

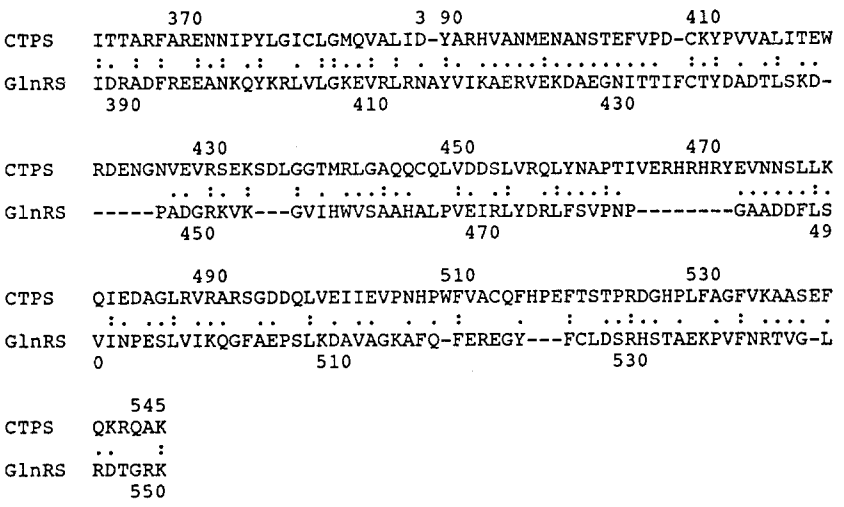

Fig. 1. This shows the alignment between the C-terminal ends of CTP synthetase (CTPS) (Weng et al. 1986) and that of the glutaminyl-tRNA synthetase (GlnRS) (Hoben et al. 1982). *A colon* indicates the same amino acid between the two sequences, while *a period* indicates a similarity score obtained using the protein matrix (Pearson and Lipman 1988). Insertions made during optimization are marked with *a dash*. See text for further information.

(Hoben et al. 1982) seems to show characteristics such as to imply a significant similarity when compared with the region containing the catalytic domain of CTP synthetase (CTPS) of *E. coli* (Weng et al. 1986), an enzyme of the *trpG* subfamily.

Figure 1 shows the alignment, obtained using the ALIGN program (see Materials and Methods), between the C-terminal end of CTPS and that of GlnRS. The compared ends are 164 amino acids long for GlnRS and 184 amino acids for CTPS. The identity percentage is 17% (32/186) and the similarity score is 58.6% (109/186). The statistical significance of this alignment (Fig. 1), established according to the RDF2 program (see Materials and Methods), is possibly significant (Lipman and Pearson 1985) with a standard deviation about 3.6 times above the mean of the optimized score, although the other scores are not significant. This behavior of statistical significance is expected for the homology discussed in this paper.

The catalytic domain of GATs of type *trpG* shows three conserved regions (Weng et al. 1986: Fig. 7) in enzymes catalyzing different reactions. At least two of these regions seem to be identifiable in Fig. 1. The first conserved segment ranges from the 373rd residue to the 390th residue of CTPS (Weng et al. 1986) and shows an identity percentage of 32% (6/19) when compared with the residues of GlnRS that range from the 398th to the 416th (Fig. 1). For the first 56 amino acids (Fig. 1) [identity percentages of 27% (15/56)] the RDF2 program gives a standard deviation about 6.2 times above the mean of the optimized score; the other scores are also significant. The second conserved segment of the CTPS catalytic domain (Weng et al. 1986) is the sequence ACQFHPE, which finds a similar sequence in GlnRS: AFQFERE (Fig. 1: from the 511th to the 517th residue of CTPS and from the 515th to the 521st residue of GlnRS). The identity percentage between these segments is 60% (4/7). Even using the consensus sequence (GFQFHPE) made up of the enzymes that possess the catalytic domain of GAT (Weng et al. 1986: Fig. 7), the identity percentage remains 60% (4/7). It is interesting to note that the alignment in Fig. 1 does not juxtapose these regions but shifts them by only six residues, indicating that although the number of amino acids separating the first conserved block of amino acids from the second block is not the same, the alignment is such as to bring these segments very close. [One hundred twenty amino acid residues separate the two conserved blocks of amino acids in CTPS vs 98 amino acids separating the similar regions in GlnRS (Fig. 1). More generally, a similar situation is observed between CTPS and the other enzymes having the catalytic domain of GATs (Weng et al. 1986: Fig. 7).] If these segments are juxtaposed, the identity of the whole C-terminal ends compared (Fig. 1) increases from 32/186 to 35/188. [If a multiple alignment of the sequences is built (Higgins and Sharp 1988, 1989) using the GlnRS sequences of *E. coli, S. cerevisiae,* and CTPS, we observe the reconstruction of this second conserved segment of GATs in the multiple alignment (data not shown).]

As regards the third region conserved between the GAT enzymes (first conserved segment in Fig. 7 in Weng et al. 1986), this does not seem to correspond to a similar region in the GlnRS sequence even if a glycine [highly conserved in the catalytic domain of these enzymes (Weng et al. 1986: Fig. 7)] at the 351st position of CTPS seems to correspond to a homologous glycine at the 375th amino acid position of GlnRS. Analogous considerations must be made for glycine at the 360th position of CTPS and for the glycine at the 383rd position of GlnRS, as the first glycine residue is conserved, for instance, between CTPS and anthranilate synthetase (Yamauchi et al. 1990: Fig. 5B). This region is not shown in Fig. 1.

Finally, there is a region of 26 amino acids that

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                490                       510
CTPS    LKQIEDAGLRVRARSGDDQLVEIIEVPNHPWFVACQFHPEF
        ::.:. .. :  : .. . . : : :: :.. ..::
GlnRS   LKDINPESEVVYKESVMEHNFGDV-VKNSPWVVDSVKNSEF
             730                  750
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Fig. 2. This shows the alignment between a stretch of the C-terminal ends of CTP synthetase of *E. coli* (CTPS) (Weng et al. 1986) and a stretch of glutaminyl-tRNA synthetase (GlnRS) of *S. cerevisiae* (Ludmerer and Schimmel 1987). *A colon* indicates the same amino acid between the two sequences while *a period* indicates a similarity score obtained using the protein matrix (Pearson and Lipman 1988). Insertion made during optimization is marked with *a dash*. See text for further information.

has an identity percentage of 23% (6/26) between CTPS and GlnRS (Fig. 1: from the 436th residue to the 461st residue of CTPS and from the 454th residue to the 479th residue of GlnRS) and that shows a fairly significant similarity when analyzed using the RDF2 program (about 4.2 standard deviations above the mean of the optimized score; the other scores show standard deviations of about 3.6). These amino acids are not a conserved region of the GAT catalytic domain and thus seem to represent a useful control of the similarity on the other regions that have conserved segments of GATs.

It may be expected (although with some reserve—see Discussion) that a similarity equivalent to the one shown in Fig. 1 must exist between the CTPS and GlnRS of the eukaryotes. To this end, using the ALIGN program, I have aligned the C-terminal end of CTPS (Fig. 1) with that of GlnRS of *S. cerevisiae* (Ludmerer and Schimmel 1987), obtaining an identity percentage of 10%, which is clearly not significant. However, using the LFASTA program I have compared the C-terminal ends of CTPS (Fig. 1) with the whole GlnRS sequence (809 amino acids) of *S. cerevisiae*. This program identifies an overlap region of 45 amino acids with an identity percentage of 20% (9/45). A further optimization of this alignment gives the result shown in Fig. 2. [The identity percentage is 29% (12/41) (Fig. 2). The standard deviation is 3.9 above the mean of the optimized score. The other scores show standard deviations of 4.1.] The data are interesting as they seem to indicate that the C-terminal end of GlnRS of an eukaryote also conserves vestiges of similarity with the C-terminal end of CTPS. The important fact is that the alignment in Fig. 2 is compatible with that in Fig. 1 and was obtained under conditions that did not force the latter alignment. Moreover, this alignment (Fig. 2) is due only partly to the matches existing between the GlnRS of *E. coli* and that of *S. cerevisiae* as only 4 of the 12 matches shown in Fig. 2 are also matches between the GlnRSs belonging to these two organisms. Therefore, Fig. 2 seems to confirm the statistical significance of the alignment in Fig. 1 and also appears to make the latter alignment less fragmented. If this is considered more carefully, it might be the very type of similarity (Fig. 2) we expect because the Glu-tRNA^{Gln} → Gln-tRNA^{Gln} pathway might have been abandoned by eukaryotes before eubacteria, as some of the latter still conserve it. Therefore, this would seem to reflect a later abandonment of the GAT function of GlnRS by eubacteria. This reasoning could justify the higher sequence similarity between CTPS and GlnRS of *E. coli* (Fig. 1) when compared with that between CTPS and GlnRS of *S. cerevisiae* (Fig. 2).

These observations seem to suggest that there may be a significantly similarity between the C-terminal ends of GlnRS and that of CTPS, or more generally with the GAT catalytic domain, and that therefore these regions are possibly homologous.

Discussion

The data and evolutionary arguments reported in this paper seem to point to the hypothesis of a homology between the C-terminal end of glutaminyl-tRNA synthetase (GlnRS) and that of CTP synthetase (CTPS) or, more generally, with the catalytic domain of glutamine amidotransferases (GATs) of type *trpG*. This evidence seems to confirm the existence of a primitive catalytic domain of amidotransferases (Kaplan et al. 1985; Zalkin et al. 1985; Danchin 1989).

More generally, these observations seem to give weight to the hypothesis (Ycas 1974; Jensen 1976) that the origin of enzyme specificity evolved through the recruitment of proteins that already existed in the catalyzation of similar reactions. We believe the catalytic domain of GATs very early on had a broad specificity of substratum as the domain was able to catalyze a class of reactions and that this domain was successively recruited both to build GAT enzymes of type *trpG* and at least the C-terminal end of GlnRS.

Nevertheless, it could be objected that the similarity between the C-terminal ends of GlnRS and those of CTPS (or, more generally, with the GAT catalytic domain) is a peculiar characteristic of the eubacteria, as the GAT activity is now found at least in the Gram-positive bacteria, where it replaces GlnRS (Wilcox and Nirenberg 1968; Schon et al. 1988). Furthermore, the most important, functional, part of the GlnRS (core region of the enzyme) ranges from the 25th to the 370th amino acid (Rould et al. 1989; Kaiser et al. 1992) and is therefore not part of the sequence shown in Fig. 1. It must, therefore, be shown that the C-terminal end of the GlnRS of eubacteria is also homologous at least to that of the eukaryotes. To this end, using the ALIGN program (see Materials and Methods), I have aligned the C-terminal end of the GlnRS of *E. coli* (as defined in Fig. 1) with that of *S. cerevisiae*

(Ludmerer and Schimmel 1987), obtaining an identity percentage of 31%. [The RDF2 program (see Materials and Methods) provides a standard deviation greater than 6.0 above the mean level of the optimized score; the initial score shows a standard deviation of about 12.0.] This seems to imply that the GAT catalytic domain was already present on the GlnRS molecule before the divergence between eukaryotes and eubacteria and must possibly have carried out a function. In the eukaryotes and eubacteria (excluding, for instance, Gram-positive bacteria) this function was abandoned and replaced by the core region of GlnRS.

The conclusion that seems to derive from these considerations is that not only does GlnRS seem to be a mosaic protein but, above all, its sequence contains traces that suggest it might represent a palimpsest. In other words, the C-terminal end of GlnRS does not seem to have great functional importance now (Rould et al. 1989; Kaiser et al. 1992). However, if the main result of the present paper is correct, this seems to imply that the primordial GlnRS performed the function of a GAT and that this function was abandoned and replaced by that of the modern GlnRS. The GlnRS sequence, therefore, shows the characteristics of a palimpsest. [It is highly unlikely that GlnRS now has the activity of a GAT since the amino acids involved in this catalysis (cysteine in the 379th position and histidine in the 515th position of CTPS, or, more generally, of GATs; Weng et al. 1986) do not seem to be homologous to amino acids in the GlnRS sequence (Fig. 1). This is expected if the activity of GATs is replaced by the activity of the core region of GlnRS.] Therefore, this view might imply that the transamidation pathway of the Glu-tRNA^{Gln} complex may have been the evolutionary precursor of the direct tRNA^{Gln} aminoacylation pathway and, also, that glutamyl-tRNA synthetase (the enzyme loading the tRNA^{Gln} in the organisms lacking GlnRS) has a common origin with glutaminyl-tRNA synthetase, as is supported by sequence data (Nagel and Doolittle 1991). These conclusions may cast light on which mechanism determined the organization of the genetic code (Wong 1981).

Gatti and Tzagoloff (1991) have shown data in favor of a homology between the asparagine synthetase (AS) of *E. coli*, an enzyme of the *purF* subfamily of GATs, and aminoacyl-tRNA synthetase (ARS) specific for Asp (AspRS). It is also well known that the ARSs specific for Asp, Asn, and Lys are members of a well-defined subclass of ARSs (Nagel and Doolittle 1991; Cusack et al. 1991; Moras 1992). These observations are in perfect agreement with both the results and the evolutionary hypotheses referred to in the present paper. From the point of view of the coevolution hypothesis (Wong 1975, 1981), the pair of amino acids Asp-Asn might have undergone an evolution equivalent to that of the pair Glu-Gln. The similarity between AS and AspRS, and therefore also AsnRS, regards the AS region that uses ammonia in the amination reaction which converts Asp into Asn (Gatti and Tzagoloff 1991). This seems to imply that, in the Asp → Asn transformation, ammonia was used as donor of the NH_2 group, and this may have a more precise prebiotic meaning than the use of glutamine, which is also used in this reaction to donate NH_2 groups. More specifically, the evolutionary precursor of the pathway of direct aminoacylation of tRNA^{Asn} might have been the amination of Asp with NH_3 while it was loaded on a tRNA-like molecule. In evolutionary terms, this seems to be equivalent to what has already been observed for Glu-Gln.

More generally, these observations must be important in establishing the origin of all ARSs. In the study of their primary structure ARSs have been divided into two classes (Eriani et al. 1990; Nagel and Doolittle 1992; Cusack et al. 1991). The results of this paper and that of Gatti and Tzagoloff (1991) suggest that the class I ARSs are homologous to the GATs of the *trpG* subfamily (Weng and Zalkin 1987) while those in class II are homologous to the GATs of the *purF* subfamily (Mei and Zalkin 1989). The conclusion that seems to derive from this is that, very early on, ARSs were two peptides capable of catalyzing two simple reactions that led to the biosynthesis of two amino acids. This generalizes our working hypothesis and might be the starting point for further investigations aiming to understand the origin of protein synthesis.

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