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tRNA specificity of a mischarging aminoacyl-tRNA synthetase: glutamyl-tRNA synthetase from barley chloroplasts

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Glutamyl-tRNA synthetase (GluRS) from barley chloroplasts is a naturally occurring mischarging enzyme: it efficiently aminoacylates both the homologous $tRNA^{Glu}$ as well as $tRNA^{Gln}$ species. In order to shed light on the structural requirements of this enzyme for tRNA specificity heterologous charging experiments were performed. Sequence comparison of tRNAs recognized by barley chloroplast GluRS reveals that two base pairs, one in the acceptor stem and one in the anticodon stem, appear to be critical for substrate recognition.

tRNA; Glutamyl-tRNA synthetase; Misaminoacylation; Substrate specificity; Chloroplast

1. INTRODUCTION

Chloroplasts contain a complete protein synthesizing system different from that found in the cytosol of plant cells. Comparative studies of aminoacyl-tRNA synthetases from organelles and cytoplasm should provide interesting data on tRNA specificity, as it is well known that there is a lack of universal cross-charging of organelle tRNAs by cytoplasmic enzymes and vice versa [1-4]. We recently showed that, in contrast to the situation in the cytoplasm, barley chloroplasts do not contain a glutaminyl-tRNA synthetase (GlnRS) activity. Rather, the formation of Gln-$tRNA^{Gln}$ required for chloroplast protein synthesis proceeds through the intermediate generation of Glu-$tRNA^{Gln}$ by chloroplast glutamyl-tRNA synthetase (GluRS) and the subsequent transamidation of the glutamate attached to $tRNA^{Gln}$ [5]. Thus, barley chloroplast GluRS is a naturally occurring mischarging aminoacyl-tRNA synthetase. It glutamylates all three major glutamate accepting tRNAs from barley chloroplasts. One of these is a bona fide $tRNA^{Glu}$, which functions in protein biosynthesis and in the formation of δ-aminolevulinic acid, a precursor in chlorophyll formation [6]. The two other glutamate acceptors are differentially modified forms of chloroplast $tRNA^{Gln}$. They are efficiently glutamylated by aminoacyl-tRNA synthetases from chloroplasts and from *Bacillus subtilis*, but not by *Escherichia coli* enzymes [5].

The phenomenon of a mischarging GluRS is widely found in living systems [5]. For instance, it has been demonstrated in several species of gram-positive bacteria [7]. In addition, it was demonstrated that the purified *B. subtilis* GluRS is responsible for glutamylating both $tRNA^{Glu}$ and $tRNA^{Gln}$ species [8]. Since in this case GluRS shows a remarkably low substrate specificity, we were prompted to perform heterologous charging experiments with a barley chloroplast GluRS preparation and a number of glutamate accepting tRNAs of known sequence in order to determine the sequence elements crucial for substrate recognition by barley chloroplast GluRS.

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2. MATERIALS AND METHODS

Preparation of unfractionated tRNA, partial purification of

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barley chloroplast GluRS and HPLC fractionation of chloroplast tRNA were performed as described [6]. The specific activity (glutamate acceptance) of the HPLC purified chloroplast tRNAs were 107 pmol/A_{260} ($tRNA^{Glu}$), 187 pmol/A_{260} ($tRNA_1^{Gln}$) and 205 pmol/A_{260} ($tRNA_2^{Gln}$), respectively, when assayed in a homologous enzyme preparation. *E. coli* $tRNA_2^{Glu}$ (spec. act. 1200 pmol/A_{260}) was obtained from the Oak Ridge National Laboratory, *E. coli* $tRNA_1^{Gln}$ (1450 pmol/A_{260}) was purchased from Subriden RNA (Rolling Bay, WA), and purified *E. coli* $tRNA_2^{Gln}$ (1600 pmol/A_{260}) was prepared in our laboratory (Perona et al., in preparation). [^{14}C]Glutamate (288 mCi/mmol) was from Amersham. All reagents were of analytical grade.

2.1. *Aminoacylation assays*

The Blue Sepharose fraction of barley chloroplasts [6] was used throughout as an enzyme source. Aminoacylation reactions contained 100 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 4 mM β-mercaptoethanol, 5 mM ATP, 13.3 A_{260}/ml HPLC-enriched chloroplast tRNA, 33 A_{260}/ml total bacterial or chloroplast tRNA or 1.6 A_{260}/ml of the respective pure tRNA species, 25 μM [^{14}C]glutamate and 0.5 mg/ml protein; incubation was at 28°C for 20 min. Aliquots of 15 μl were spotted onto paper filter discs, washed in ice cold 10% and 5% trichloroacetic acid, rinsed in ethanol, dried and counted for radioactivity after the addition of scintillation fluid. Discrimination between $tRNA^{Gln}$ and $tRNA^{Glu}$ was made by precharging the respective total tRNA preparation with [^{12}C]glutamate or [^{12}C]glutamine (0.6 mM) in an *E. coli* extract for 10 min at 37°C. After phenol extraction and ethanol precipitation, these precharged tRNAs were used in an aminoacylation assay with the barley Blue Sepharose fraction and [^{14}C]glutamate.

3. RESULTS

The specificity of chloroplast GluRS is unique: since in chloroplasts lacking GlnRS activity [5], this enzyme has to charge efficiently two tRNAs of strikingly different sequence, namely $tRNA^{Glu}$ and $tRNA^{Gln}$. On the other hand, the barley chloroplast GluRS does not aminoacylate the homologous major cytoplasmic $tRNA^{Glu}$ species [5]. In order to obtain information about the structural requirements of a tRNA for proper recognition by barley chloroplast GluRS, we performed charging experiments with a number of glutamate- or glutamine-specific tRNAs of known sequence. The incorporation of [^{14}C]glutamate into pure or HPLC-fractionated tRNAs clearly shows that barley chloroplast GluRS efficiently charges *E. coli* $tRNA^{Glu}$ and both $tRNA^{Glu}$ and $tRNA^{Gln}$ from chloroplasts, but none of the two *E. coli* $tRNA^{Gln}$ species (table 1). The differences in charging efficiency for the glutamate-accepting, HPLC-fractionated chloroplast tRNAs can be assigned to their different degrees of purity (see section 2).

Table 1

Charging efficiencies of barley chloroplast GluRS using homologous and heterologous tRNA substrates

tRNA	Glu-tRNA per assay (pmol)	Glu-tRNA (pmol/A_{260})
E. coli Glu_2	10	500
Gln_1	0	–
Gln_2	0	–
Barley chloroplast		
Glu	21.5	107.5
Gln_1	37.5	187.5
Gln_2	41	205

Discrimination between $tRNA^{Gln}$ and $tRNA^{Glu}$ in crude tRNA preparations from *B. subtilis*, barley or spinach chloroplasts, and *Synechocystis* was possible based on the known specificity of *E. coli* GluRS and GlnRS. It has been established that only $tRNA^{Gln}$, but not $tRNA^{Glu}$ from *B. subtilis* is glutaminylated by *E. coli* synthetases [8], while unfractionated *E. coli* synthetases glutamylate $tRNA^{Glu}$, but not $tRNA^{Gln}$ from chloroplasts and *Synechocystis* [5]. We therefore precharged the respective tRNAs with unlabeled glutamine or

Table 2

Discrimination between the recognition of $tRNA^{Glu}$ and $tRNA^{Gln}$ from homologous and heterologous sources by precharging the respective total tRNA preparations with unlabeled glutamine (for *B. subtilis*) or glutamate (all others) in an *E. coli* extract and subsequent charging with [^{14}C]glutamate in a barley chloroplast extract (see section 2)

tRNA	[^{14}C]Glu-tRNA per assay (pmol)	tRNA charged ([^{14}C]Glu-tRNA)	% of untreated
Barley chloroplast			
(−)	32.5		100
(+)	17.2	$tRNA^{Gln}$	51
Spinach chloroplast			
(−)	12.2		100
(+)	5.42	$tRNA^{Gln}$	45
Synechocystis			
(−)	26.3		100
(+)	12.5	$tRNA^{Gln}$	48
B. subtilis			
(−)	22.3		100
(+)	22.7	$tRNA^{Glu}$	102

Untreated (−) and precharged (+) tRNA preparations

glutamate in an *E. coli* extract (see section 2). These precharged tRNAs were then aminoacylated with [^{14}C]glutamate by barley chloroplast GluRS (Blue Sepharose fraction). Comparison of the amount of radioactivity incorporated into the precharged tRNA to that obtained with the respective untreated tRNAs revealed that in *B. subtilis* tRNA only the $tRNA^{Glu}$ species is recognized by barley chloroplast GluRS. On the other hand, both $tRNA^{Gln}$ and $tRNA^{Glu}$ present in the unfractionated tRNA from chloroplasts and *Synechocystis* are effectively glutamylated by this enzyme (table 2). It can also be concluded from the data in this table that the glutamate- and glutamine-specific tRNAs (as defined by their anticodon sequences) are present in approximately equimolar amounts in chloroplasts and *Synechocystis*. The comparatively low specific activity of the spinach tRNA might be assigned to inaccurate determination of RNA concentration due to the presence of UV absorbing compounds in the preparation.

4. DISCUSSION

In barley chloroplasts, as in *B. subtilis* [8], a single enzyme is responsible for glutamylating both $tRNA^{Glu}$ and $tRNA^{Gln}$ [9]. The number of bases identical in barley chloroplast $tRNA^{Glu}$ and $tRNA^{Gln}$ (48% homology) makes it impossible to identify, by sequence comparison, nucleotides in tRNA that might be important for the specific recognition by GluRS. Therefore, we decided to approach this problem by examining the sequences of heterologous glutamate-accepting tRNAs based on whether they are substrates of barley chloroplast GluRS or not (tables 1 and 2). The results show that, in addition to the charging of both $tRNA^{Glu}$ and $tRNA^{Gln}$ from barley and spinach chloroplasts and *Synechocystis*, the barley chloroplast GluRS efficiently glutamylates the $tRNA^{Glu}$, but not $tRNA^{Gln}$ species from *B. subtilis* and *E. coli*. This is in contrast to the specificity of *B. subtilis* GluRS which recognizes, in addition to the homologous $tRNA^{Glu}$ and $tRNA^{Gln}$, also the $tRNA_1^{Gln}$, but not $tRNA_2^{Gln}$ and $tRNA^{Glu}$ from *E. coli* [8]. It has been suggested that in this case one base pair in the T-stem is involved in the specificity of tRNA recognition by GluRS.

Comparison of the sequences of the tRNAs that are glutamylated (i.e. barley chloroplast $tRNA^{Glu}$ and $tRNA^{Gln}$, *E. coli* $tRNA^{Glu}$ and *B. subtilis* $tRNA^{Glu}$) to those that are not charged by barley chloroplast GluRS (i.e. *B. subtilis* $tRNA^{Gln}$ as well as both $tRNA^{Gln}$ species from *E. coli*) allows one to identify putative recognition sites on the tRNA. If one eliminates all nucleotides conserved in both groups of tRNAs, as well as those conserved in one group, but also present in one or more members of the other group, then only bases or base pairs exclusively present in either group remain; thus they are probably relevant for the specificity of synthetase recognition. For all the tRNAs compared, only one base pair in the acceptor stem and one base pair in the anticodon stem meet these criteria (fig.1): all tRNAs that are efficiently glutamylated have a $U_6 \cdot G_{67}$, $C_6 \cdot G_{67}$ or $G_6 \cdot C_{67}$ and a $U_{31} \cdot G_{39}$, $G_{31} \cdot C_{39}$ or $C_{31} \cdot G_{39}$, whereas the tRNAs that are not recognized have an $A_6 \cdot U_{67}$ pair and a $U_{31} \cdot A_{39}$ or $A_{31} \cdot U_{39}$ pair in these two positions. The primary structure of *Synechocystis* 6803 $tRNA^{Glu}$

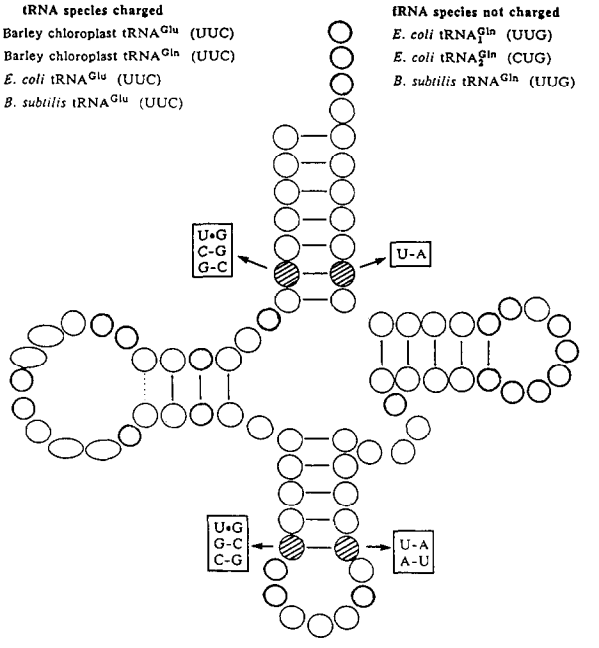

Fig.1. Cloverleaf diagram of tRNA illustrating the positions of the base pairs important for recognition by chloroplast GluRS. The base pairs (positions $N_6 \cdot N_{67}$ and $N_{31} \cdot N_{39}$) found in tRNA species that are charged by chloroplast GluRS are listed to the left of the cloverleaf diagram, the base pairs found in the non-recognized tRNAs in these positions are listed to the right.

(O'Neill et al., in preparation), as well as the published chloroplast $tRNA^{Glu}$ and $tRNA^{Gln}$ gene sequences [10,11], also meet these criteria. Thus, we conclude that the $N_6 \cdot N_{67}$ and $N_{31} \cdot N_{39}$ base pairs play an important role in substrate recognition by barley chloroplast glutamyl-tRNA synthetase. This hypothesis will be tested by experiments involving in vitro mutagenesis of the respective cloned tRNA genes.

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REFERENCES

[1] Reger, B.J., Fairfield, S.A., Epler, J. and Barnett, W.E. (1970) Proc. Natl. Acad. Sci. USA 67, 1207–1213.
[2] Kislev, N., Selsky, M.I., Norton, C. and Eisenstadt, J.M. (1972) Biochim. Biophys. Acta 287, 256–269.
[3] Martin, R.P., Sibler, A.P., Bordonne, R. and Dirheimer, G. (1983) Mol. Biol. 17, 1126–1146.
[4] Dietrich, A., Souciet, G., Colas, B. and Weil, J.H. (1983) J. Biol. Chem. 258, 12386–12393.
[5] Schön, A., Kannangara, C.G., Gough, S. and Söll, D. Nature, submitted.
[6] Schön, A., Krupp, G., Gough, S., Berry-Lowe, S., Kannangara, C.G. and Söll, D. (1986) Nature 322, 281–284.
[7] Wilcox, M. and Nirenberg, M. (1968) Proc. Natl. Acad. Sci. USA 61, 229–236.
[8] Lapointe, J., Duplain, L. and Proulx, M. (1986) J. Bacteriol. 165, 88–93.
[9] Bruyant, P. and Kannangara, C.G. (1987) Carlsberg Res. Commun. 52, 99–109.
[10] Sprinzl, M., Hartmann, T., Meissner, F., Moll, J. and Vorderwülbecke, T. (1987) Nucleic Acids Res. 15, r53–r188.
[11] Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H. and Ozeki, H. (1986) Nature 322, 572–574.