THE ROLE OF THE ANTICODON REGION IN HOMOLOGOUS AND HETEROLOGOUS CHARGING OF $tRNA^{Phe}$

R. THIEBE AND H.G. ZACHAU

Institut für Physiologische Chemie der Universität München, Germany

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In the nucleotide sequence of $tRNA^{Phe}$ from yeast, which has been established by RajBhandary, Khorana and their coworkers[1], the nucleoside Y is located right next to the anticodon. The structure of Y is still unknown. We recently observed[2] that the base moiety of Y can be excised from $tRNA^{Phe}$ by a mild treatment with acid without breaking the polynucleotide chain. The modified tRNA which was called $tRNA^{Phe}_{HCl}$ does not differ from $tRNA^{Phe}$ in its acceptor capacity for phenylalanine when a phenylalanyl tRNA synthetase from yeast is used for charging, but is not bound at all to polynucleotide ribosome complexes. It was now found that $tRNA^{Phe}_{HCl}$ in contrast to $tRNA^{Phe}$ is not charged by synthetases from E. coli. Related phenomena were observed with tRNA preparations from wheat germ and rat liver.

Materials and Methods: Unfractionated tRNA from brewer's yeast ("$tRNA_{yeast}$") and purified phenylalanine specific tRNA from yeast ("$tRNA^{Phe}$") were prepared as previously described[2]. The acid induced elimination[2] of the base moiety of Y (standard conditions, 3 hours at pH 2.9 and 37°) was carried out with $tRNA_{yeast}$ and $tRNA^{Phe}$ resulting in "$tRNA_{HCl}$" and "$tRNA^{Phe}_{HCl}$", respectively. "$tRNA_{coli}$" was purchased from General Biochemicals Chagrin Falls, Ohio (Lot. 640017, prepared from E. coli B). Rat liver tRNA was prepared by conventional

methods. Wheat germ tRNA was a gift of Dr. I. Svensson, Uppsala. Aminoacyl tRNA synthetase fractions were prepared from E. coli B and K12 CR63 analogously to the ones from yeast[2]. 8 fold and 340 fold purified preparations of phenylalanyl tRNA synthetase from E. coli N.P.3 $B1^-$ RC^{rel} (previously K10) were gifts of Dr. A. Böck, who had prepared them according to his modifications[3] of the procedure of Fangman and Neidhardt[4]. The conditions of charging were as previously described[2]. Incubation mixtures of 0.1 ml contained 0.5 or 0.035 A_{260} units of tRNA (Fig. 1 and 2 respectively), 0.1 A_{280} unit of the yeast synthetase or 0.3 A_{280} units of the synthetase from E. coli B or E. coli K12. In Fig. 1 the amounts of synthetase from E. coli K10 (in enzyme units[4]) were identical to those of E. coli B synthetase. In Fig. 2 the relative amounts of enzyme (in enzyme units[4]) were 1:2:3.5 for the synthetases from E. coli K10 (8 fold), B, and K10 (340 fold), respectively. All incorporations of ^{14}C-phenylalanine could be suppressed by addition of non-radioactive phenylalanine.

Results: $tRNA_{yeast}$ was charged only partially by the crude synthetase fractions from E. coli (Fig. 1). In the presence of 0.25 µmoles spermidine per 0.1 ml of incubation mixture, however, the level of homologous charging was reached within 15 minutes. The charging of $tRNA_{coli}$ by the synthetase from yeast was very low but significantly above background. $tRNA_{HCl}$ was charged by the homologous yeast synthetase as well as the untreated $tRNA_{yeast}$; with the synthetase from E. coli B, on the other hand, only a small amount of phenylalanine was accepted. Since $tRNA_{HCl}$ still contained some unchanged phenylalanine specific

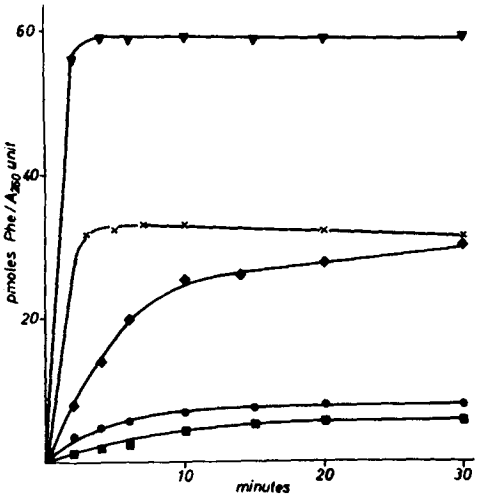

Fig. 1: Charging of unfractionated tRNA: ▼ $tRNA_{yeast}$, synthetase from yeast (an identical curve was obtained with $tRNA_{HCl}$); × $tRNA_{coli}$, synthetase from E. coli B (an identical curve was obtained with synthetase from E. coli K10, 8 fold; incorporations with synthetase from E. coli K12 were 15% lower); ◆ $tRNA_{yeast}$, synthetase from E. coli B; ● $tRNA_{HCl}$, synthetase from E. coli B; ■ $tRNA_{coli}$, synthetase from yeast.

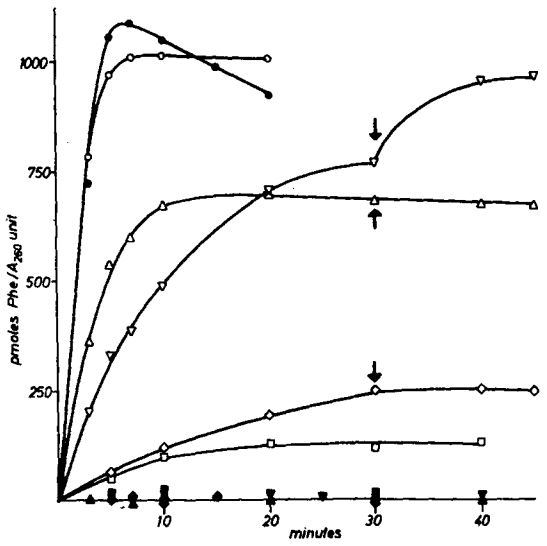

Fig. 2: Charging of purified $tRNA^{Phe}$ and $tRNA^{Phe}_{HCl}$: Open symbols $tRNA^{Phe}$, filled symbols $tRNA^{Phe}_{HCl}$. ○ ● synthetase from yeast; ◇ ◆ synthetase from E. coli B; □ ■ synthetase from E. coli K12; ▽ ▼ synthetase from E. coli K10 (8 fold); △ ▲ synthetase from E. coli K10 (340 fold). Arrows indicate a second addition of synthetase.

$tRNA_{yeast}$, the incorporation can be attributed to this contamination. Purified $tRNA^{Phe}_{HCl}$, which accepted more than 1000 pmoles phenylalanine/A_{260} unit with synthetase from yeast, was not charged at all with 4 different synthetase preparations from E. coli (Fig. 2). (The decrease in the extent of

homologous charging of $tRNA^{Phe}_{HCl}$ at longer times was a reproducible phenomenon, which is not explained; it may be attributed to a greater lability of this tRNA in the charging mixtures with the rather crude synthetase.) $tRNA^{Phe}$ was charged by the synthetase from E. coli K10 (340 fold) to an extent of 70%. A second addition of synthetase at 30 minutes did not increase the incorporation while added $tRNA^{Phe}$ was still charged. Since the 8 fold purified synthetase from E. coli K10 gave 97% charging of $tRNA^{Phe}$, the incomplete charging with the highly purified synthetase may be attributed to the lack of terminal A in part of $tRNA^{Phe}$ and the absence of CCA-pyrophosphorylase in this synthetase preparation. The low incorporation into $tRNA^{Phe}$ by synthetases from E. coli B and K12 should be due to secondary effects as ribonuclease contaminations since unfractionated $tRNA_{yeast}$ was charged rather well at least by the synthetase from E. coli B.

The initial rates of charging of $tRNA_{yeast}$ and $tRNA^{Phe}$ by synthetase from E. coli B were 6%; with synthetase from E. coli K10 (340 fold) 5 and 10%, respectively, were observed ($tRNA_{coli}$ 100%). The Michaelis constant of the synthetase from E. coli K10 (8fold) for $tRNA^{Phe}$ was 8×10^{-7}; no effect on the kinetics of charging was observed when $tRNA^{Phe}_{HCl}$ was added.

Phenylalanine specific tRNAs from rat liver and wheat germ as well as their respective acid conversion products were purified by a series of chromatographies on benzoylated DEAE-cellulose columns (analogously to $tRNA^{Phe}$ and $tRNA^{Phe}_{HCl}$ from yeast[2]). The synthetase from yeast charged all 4 tRNAs equally well, while the synthetase preparations from E. coli K10 charged only the

2 unmodified tRNAs. The synthetase from E. coli B charged only the unmodified tRNA from rat liver.

Discussion: Charging of $tRNA_{yeast}$ with phenylalanine by synthetase from E. coli has been observed previously[5,6] and the effect of spermine on this charging has been studied[5]. Heterologous charging with valine is known to proceed slower than charging with homologous synthetases[7]. Experiments from a number of groups including ours, which have been compiled and discussed recently[2], demonstrate that the anticodon regions of various tRNAs can be modified without loss of acceptor activity in homologous charging reactions. We were therefore surprised to find that in heterologous charging reactions with synthetases from E. coli, excission of the base moiety of Y from tRNAs of 3 different species abolishes their phenylalanine acceptance. At least in the $tRNA^{Phe}$ of yeast[1] and wheat germ[8] Y is located in the anticodon region. Particular structural features of Y, which would be required for recognition by the synthetase from E. coli, could not be the reason for the effect since $tRNA_{coli}$ does not contain Y[2]. Therefore a change in the conformation of the anticodon region has to be invoked which is in agreement with previous conclusions from UV measurement[2]. ORD spectra of $tRNA^{Phe}$ and $tRNA^{Phe}_{HCl}$ from yeast, measured by Dr. J. Engel, do not exclude a small change in conformation. The change in the anticodon region may influence the conformation of other parts of the tRNA molecules. Heterologous charging seems to offer a sensitive tool for detecting structural changes in tRNAs.

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