CORRECTION OF AMINOACYLATION ERRORS: EVIDENCE FOR A NON SIGNIFICANT ROLE OF THE AMINOACYL-tRNA SYNTHETASE CATALYSED DEACYLATION OF AMINOACYL-tRNAs

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Received 1 January 1974

1. Introduction

It has been reported that aminoacyl-tRNAs can be deacylated by their cognate aminoacyl-tRNA synthetase in the absence of AMP and pyrophosphate [1–8]. Some authors [4, 8, 9] postulated that this reaction has a physiological role by correcting aminoacylation errors. Indeed, some wrong aminoacyl-tRNAs are deacylated 100 times more rapidly than the correct ones [7–9]. On the other hand, some other wrong aminoacyl-tRNAs are very slowly deacylated [7–9].

In this work, we bring new data about the deacylation of correctly and incorrectly charged yeast $tRNA^{Met}$ and $tRNA^{Ala}$ by alanyl-, valyl- and phenylalanyl-tRNA synthetases. These data, added to previously reported ones concerning the deacylation of various aminoacyl-$tRNAs^{(Phe, Met, Val)}$ [7–10] led us to think that this reaction plays a negligible role in the correction of aminoacylation errors.

2. Materials and methods

Phenylalanyl-tRNA synthetase (PRS) was prepared by F. Fasiolo [11]. Valyl-tRNA synthetase (VRS) and alanyl-tRNA synthetase were prepared by D. Kern [12]. The purification of methionyl-tRNA synthetase (MRS) performed by D. Kern will be reported elsewhere. $tRNA^{Phe}$ was purified by counter-current distribution [13], $tRNAs^{(Val, Met, Ala)}$ were purified by the same counter-current distribution followed by BD-cellulose and RPC5 chromatography.

Incorrect and correct aminoacylations as well as the deacylation reaction were performed as previously described [7, 14, 15].

3. Results

The following mischarged species were used: $Val\text{-}tRNA^{Met}$, $Phe\text{-}tRNA^{Met}$, $Val\text{-}tRNA^{Ala}$, $Phe\text{-}tRNA^{Ala}$. In most cases, the mischarging reaction was not complete (the yields varied from 10% to 100%). Thus the measured rate of enzymic deacylation had to be corrected by taking into account the amount of inhibition due to the uncharged tRNA. We assumed that the affinities of the uncharged and of the charged tRNAs for the aminoacyl-tRNA synthetases are identical, as suggested by several observations showing that they are either identical or very close to each other [16–18]. Nevertheless, we cannot exclude that these calculated values are slightly erroneous. These approximations are quite sufficient because we are only looking for large differences between the deacylation rates of correctly and incorrectly charged tRNAs.

Table 1 summarizes the results concerning the deacylation of correctly or incorrectly charged tRNAs by alanyl-, methionyl-, valyl- and phenylalanyl-tRNA synthetases. The rates of deacylation are rather low except for $Val\text{-}tRNA^{Phe}$ and there is no significant increase in the rate of deacylation of mischarged species. Moreover, there is no correlation between the nature of the amino acid and the rate of deacylation for a

Table 1

Rates of enzymatic deacylation of correctly and incorrectly charged aminoacyl-tRNAs by the cognate or non cognate aminoacyl-tRNA synthetases. The rates are expressed in nM/min/mg of enzyme.

Aminoacyl-tRNA	Rates of deacylation by				Rate of correct aminoacylation	tRNA or aminoacyl-tRNA concentration
	Alanyl-tRNA synthetase	Methionyl-tRNA synthetase	Phenylalanyl tRNA synthetase	Valyl-tRNA synthetase		
Phe-tRNA^{Phe}			6.8		930	13×10^{-7} M
Val-tRNA^{Phe}			850			13×10^{-7} M
Val-tRNA^{Val}				2.1*	310	13×10^{-7} M
Phe-tRNA^{Val}				0.6*		13×10^{-7} M
Met-tRNA^{Met}	0.61	0.72**	1.1	0.2		$5-7 \times 10^{-7}$ M
Phe-tRNA^{Met}	0.20	0.50**	0.2	0.45		$5-7 \times 10^{-7}$ M
Val-tRNA^{Met}	1.6	0.05**	0.08	0.25		$5-7 \times 10^{-7}$ M
Ala-tRNA^{Ala}	11.0		0.03	0.5		$5-7 \times 10^{-7}$ M
Phe-tRNA^{Ala}	0.3		0.0	0.2		$5-7 \times 10^{-7}$ M
Val-tRNA^{Ala}	0.08		0.08	0.4		$5-7 \times 10^{-7}$ M

* Data from [7].
** Data from [10].

given enzyme. This rate is a function of both the amino acid and tRNA moieties.

4. Discussion

The results show that the greatest deacylation rate is that of Val-tRNA^{Phe} by PRS. It nearly reaches the rate of aminoacylation. This is also the case for the deacylation of Val-tRNA^{Ile} from *E. coli* by isoleucyl-tRNA synthetase [8] and for Ile-tRNA^{Phe} by PRS from *E. coli* [9]. However, our results show that, in most cases, this rate of deacylation is at least 100 to 1000 times lower than that of aminoacylation.

We examined the way in which deacylation can alter the ratio between the concentration of correctly and incorrectly charged aminoacyl-tRNA (this ratio being our definition of the specificity). For this purpose we chose the system of aminoacylation of tRNA^{Phe} by PRS and VRS because the enzymic deacylation of Val-tRNA^{Phe} is at least 100-fold higher than that of other incorrectly charged tRNAs. The deacylation reaction will affect the ratio between Phe-tRNA^{Phe} and Val-tRNA^{Phe} very differently according to the situations in which this system works.

In this study we used the kinetic data previously determined for the following reactions:

- aminoacylation of tRNA^{Phe} by VRS [10] : K_M = 14 μM, k = 0.0035 min^{-1}
- aminoacylation of tRNA^{Phe} by PRS [11] : K_M = 0.2 μM, k = 300 min^{-1}
- deacylation of Val-tRNA^{Phe} by PRS [20] : K_M = 0.2 μM, k = 3 min^{-1}
- spontaneous deacylation of Phe-tRNA^{Phe} [20] : k = 0.02 min^{-1}
- spontaneous deacylation of Val-tRNA^{Phe}. We assumed that this rate of deacylation is the same as that of deacylation of Val-tRNA^{Val} [16], because it has been shown [23] that the spontaneous deacylation essentially depends on the nature of the amino acid. Therefore we took k = 0.006 min^{-1}.
- deacylation of Val and Phe-tRNA^{Phe} by VRS. This deacylation is negligible. Indeed the tRNA and enzyme concentrations used were close to those existing in the yeast cell [19] : VRS = PRS 0.2 μM; tRNA^{Val} = tRNA^{Phe} = 1 μM. In the absence of tRNA^{Val}, deacylation of Phe-tRNA^{Phe} by VRS is three times lower than PRS [7]. In the presence of tRNA^{Val}, VRS deacylates this aminoacyl-tRNA

Table 2
Influence of the deacylation upon the specificity. The specificity is defined at a given time as the ratio: $\frac{[\text{correctly charged tRNA}]}{[\text{incorrectly charged tRNA}]}$.

Condition used	Calculated specificity without taking into account the enzymatic deacylation	Calculated specificity taking into account the enzymatic deacylation of Val-tRNA^{Phe}	Calculated specificity taking into account a deacylation rate of $\frac{1}{100}$ deacylation of Val-tRNA^{Phe}
Initial velocity of aminoacylation	2.6×10^8	2.6×10^8	2.6×10^8
At equilibrium	5.9×10^6	2.9×10^9	2.6×10^7
At equilibrium in a system including an in vitro protein synthesis	2.7×10^7	9×10^7	2.7×10^7

150 times more slowly than PRS does. Consequently, this reaction can be neglected. In the same way, deacylation of Phe-tRNA^{Phe} by VRS is 10^5 times slower than by PRS. We studied the influence of the deacylation reaction upon the specificity in 3 different situations (table 2).

4.1. *Systems working in conditions of initial velocity*

Concerning initial velocities, the concentration of aminoacyl-tRNA is negligible and the ratio between the velocities of the aminoacylation of the tRNA^{Phe} by VRS and PRS cannot be affected by the deacylation phenomenon. Here, we calculated approximately the specificity using the Michaelis' law and taking into account the competition between tRNA^{Val} and tRNA^{Phe} by VRS. We found a value of 2.6×10^8 for the specificity.

4.2. *System at the equilibrium*

After a sufficient length of time, the reaction stops. We previously showed [16] that the extent of aminoacylation results from an equilibrium between the aminoacylation reaction and the spontaneous and enzymic deacylation and that the plateau level can be calculated if the kinetic parameters are known. In our system, the calculated specificity, without taking into account the enzymic deacylation is found to be 6×10^6. If the enzymic deacylation is taken into account, the specificity value will be 2.6×10^9. There is a 450-fold increase of the specificity in consequence of the high rate of enzymic deacylation of Val-tRNA^{Phe}. However this increase of specificity is rather weak when compared to the high value of specificity found without taking into account the effect of the deacylation of incorrectly charged tRNA. Moreover, we have seen that, in most cases, the rate of enzymic deacylation of incorrectly charged tRNAs is at least 100 times lower than that found for Val-tRNA^{Phe}, so that the increase of specificity due to the deacylation reaction can in most cases be considered as insignificant.

4.3. *System in the presence of in vitro protein synthesis*

In vivo, there is an equilibrium between the aminoacylation, the spontaneous deacylation, the enzymic deacylation and the incorporation of the amino acid into the proteins. It has been shown [21] that the kinetics of amino acid incorporation into proteins in an in vitro system can be written as:

$$v = \frac{V_{max} \times [\text{aatRNA}]}{K + [\text{aatRNA}]},$$

the value of K being similar to the K_M value fo the aminoacyl-tRNA synthetase for the tRNA (we took $K = 0.2\ \mu M$). On the other hand, in the yeast cell 60 to 80 per cent of the tRNAs are aminoacylated [19]. In these conditions, our calculations show that a twofold decrease in the PRS concentration leads to a 6% decrease in the rate of protein synthesis. This is in good agreement with an observation of Neidhardt who showed that in yeast cells protein synthesis drops

significantly only when the concentration of VRS is decreased two or three times.

Without taking into account the enzymic deacylation, the calculated specificity was found to be 2.7×10^7 and it becomes 9×10^7 if we take into account this deacylation. This increase of specificity cannot be considered as significant. This suggests that in this model the enzymic deacylation reaction has no appreciable role. In conclusion, it seems that in vivo this process is probably not involved in a correction mechanism of aminoacylation.

Acknowledgements

We are grateful to Dr. Giége for the preparation of incorrectly charged aminoacyl-tRNAs and to Dr. Dirheimer for advice concerning this manuscript.

This work was supported by the CNRS (laboratories associé 119), the Délégation Générale à la Recherche Scientifique et Technique (action concertée interactions moléculaires en Biologie) and by the Commissariat à l'Energie Atomique.

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