YEAST PHENYLALANYL-tRNA SYNTHETASE: EVIDENCE FOR THE TRIGGERING OF AN AMP–ATP EXCHANGE BY tRNA

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Received 28 October 1975

1. Introduction

We recently reported [1] some results favouring the existence, in the aminoacylation reaction of $tRNA^{Phe}$ by yeast phenylalanyl-tRNA synthetase (PRS), of an intermediate complex which might be different from the aminoacyladenylate–enzyme complex. If a covalent acylenzyme were involved in the transfer of the amino acid moiety from the aminoacyladenylate to the tRNA, it should be possible to observe an AMP–ATP exchange during the course of the reaction, according to the equations:

$$\text{ATP + Phe + PRS} \rightleftharpoons \text{Phe-AMP-PRS + PP}_i$$

$$\text{PHE-AMP-PRS} \rightleftharpoons \text{Phe-X-PRS + AMP}$$

(X)

Such an echange has never been observed in the absence of tRNA. Of course, in the presence of tRNA, an AMP–ATP exchange can easily be observed, but this does not demonstrate the existence of an intermediate acylenzyme, since the exchange can be explained by the overall reverse reaction. The general failure to observe the AMP–ATP exchange in the absence of tRNA might be due to the fact that this ligand is required in order to induce a conformational change of the protein, thus triggering the formation of the covalent aminoacyl-enzyme complex.

In this paper, we show that a non acylable modified $tRNA^{Phe}$, in which the terminal adenosine has been replaced by 2′-deoxyadenosine is able to induce an AMP–ATP exchange.

2. Materials and methods

The yeast phenylalanyl-tRNA synthetase was prepared by the procedure previously described [2]. Purified yeast $tRNA^{Phe}$ was obtained by countercurrent distribution, as described by Dirheimer and Ebel [3]. 2′-deoxy ATP was obtained from Sigma. All other reagents used were analytical grade chemicals from Merck, Fluka and Prolabo. Radiochemicals were purchased from the Commissariat à l'Energie Atomique - Saclay-France.

2.1. *Preparation of $tRNA^{Phe}ox$*

$tRNA^{Phe}$ (4 mg/ml) was treated with 10 mM sodium metaperiodate in 100 mM acetate buffer pH 5.0, 10 mM $MgCl_2$, during two hours at room temperature and in the dark. The reaction was stopped by addition of 1 ml of glycerol and the tRNA solution was extensively dialysed against distilled water.

2.2. *Preparation of $tRNA^{Phe}$ 2′-deoxy A*

$tRNA^{Phe}$-pCpC was prepared according to Uziel and Khym [4] by periodate oxydation at alkaline pH followed by treatment with a phosphomonoesterase. 2′-deoxyadenosine was added to the 3′ end of $tRNA^{Phe}$ pC-pC according to the method described by Sprinzl et al. [5]. In order to eliminate the normal $tRNA^{Phe}$ which could result from a contamination of 2′-deoxy ATP by normal ATP, the $tRNA^{Phe}$ 2′-deoxy A synthesized was further oxidised by sodium periodate.

2.3. *Control of the accepting capacity of native or modified $tRNA^{Phe}$*

tRNA acylation was performed under the following

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conditions: tRNA (10^{-5} M) was incubated at 37°C in 50 mM Tris–HCl buffer pH 7.8, 15 mM Mg^{++}, 10 mM ATP, 0.15 mM ^{14}C-Phe (50 μCi/μmol), $5 \cdot 10^{-4}$ M 2-mercaptoethanol, 0.1 mM EDTA, 10^{-9} M PRS. 40 μl aliquots were withdrawn at given time intervals and applied to Whatman 3 MM paper discs which were immediately soaked in 5% trichloracetic acid (w/v). The discs were further washed as described by Mans and Novelli [6].

2.4. *AMP–ATP exchange reaction*

The reaction mixture had the following composition: 100 mM Hepes buffer pH 7.0, 4 mM ATP, 4 mM Phe, 0.4 mM [^{14}C]AMP (46 μCi/nmol), 0.015 mM $tRNA^{Phe}$ or $tRNA^{Phe}$-2′-deoxy A, 20 mM Mg^{++}, 0.6 mM sodium pyrophosphate, 3 μM PRS. The mixture was incubated at 37°C and the reaction was initiated by addition of the enzyme. At given time intervals, 20 μl aliquots were withdrawn, mixed with 20 μl of ethanol to stop the reaction and kept in ice.

AMP and ATP in the different aliquots were separated by thin layer chromatography on Silicagel plates (Merck F254) in the solvent $CH_3OH–NH_4OH–H_2O$, (60:10:20, v/v/v) as previously described [7]. Non-labelled AMP and ATP were also spotted on the plates to act as markers. Spots were visualised under u.v. light, scratched and the radioactivity was measured by liquid scintillation.

3. Results and discussion

As can be seen on fig.1, in the presence of $tRNA^{Phe}$, the AMP–ATP exchange, resulting from the overall reverse reaction is easily detected (30% of the total radioactivity is incorporated in ATP per min of incubation). On the contrary in the absence of tRNA, only a very faint AMP–ATP exchange can be detected (1.3% of the total radioactivity is incorporated inATP per hour of incubation). Thus the initial rate of AMP–ATP exchange

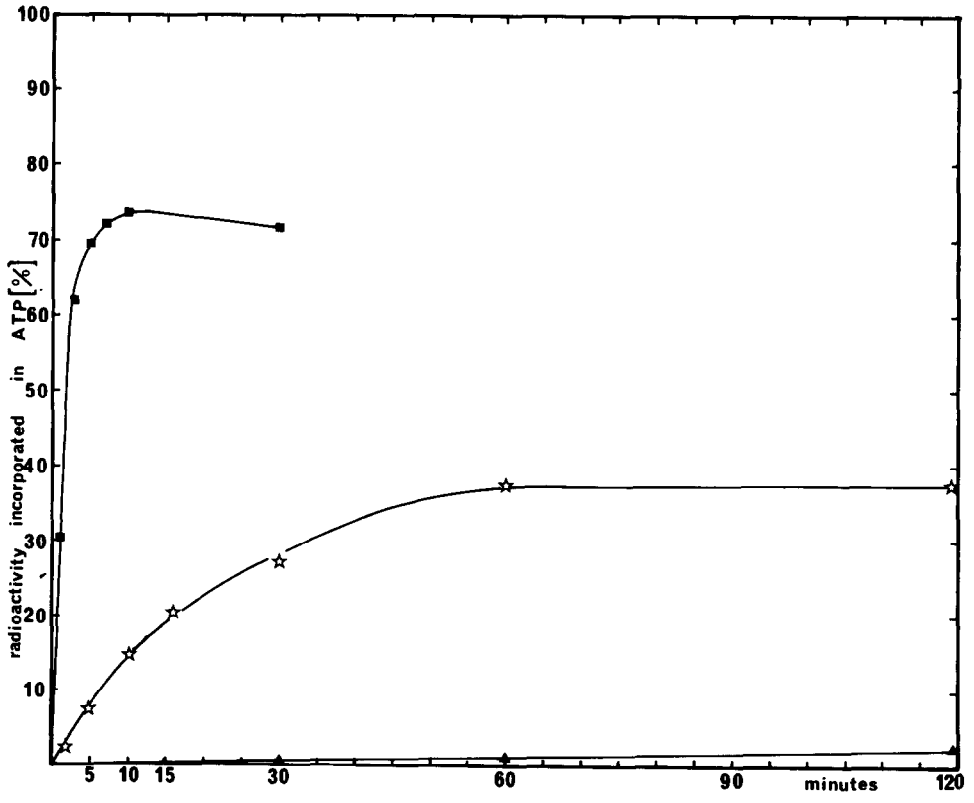

Fig.1. AMP–ATP exchange as a function of time. (▲——▲) During the activation of Phe. (■——■) In the course of $tRNA^{Phe}$ acylation. (☆——☆). In the presence of $tRNA^{Phe}$ 2′-deoxy A.

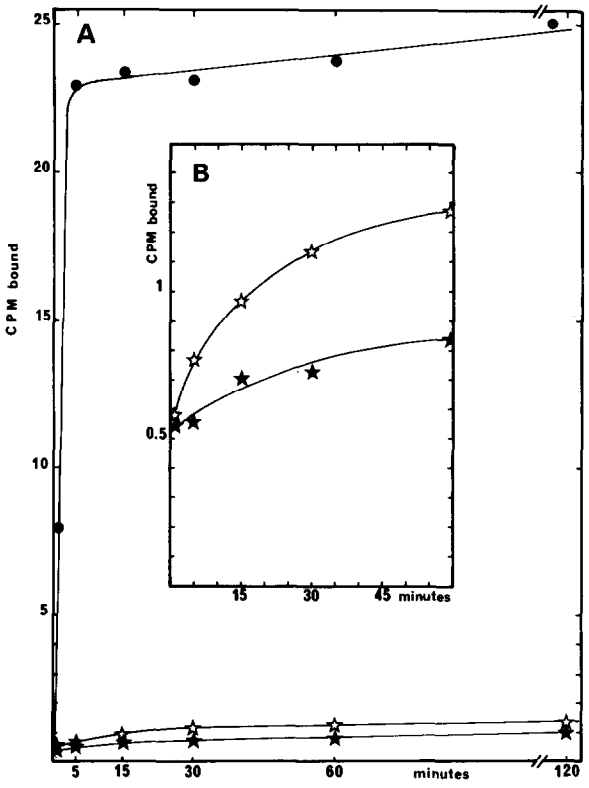

Fig.2. (A) Kinetics of acylation of: (●——●) $tRNA^{Phe}$. (☆——☆) $tRNA^{Phe}$ 2′-deoxy A. (★——★) $tRNA^{Phe}$ ox. (B) Insert shows the residual acylations of $tRNA^{Phe}$ 2′-deoxy A (☆——☆) and $tRNA^{Phe}$ ox (★——★) with a 10-fold expanded ordinate scale.

in the absence of tRNA corresponds to about 1/1400 of the AMP–ATP exchange due to the overall reverse reaction. The addition of $tRNA^{Phe}$ 2′-deoxy A strongly stimulates the AMP–ATP exchange, since in the presence of this modified tRNA, 1.5% of the total radioactivity is incorporated in ATP per minute of incubation, which corresponds to a 70-fold stimulation.

Obviously, it has to be ascertained that $tRNA^{Phe}$ 2′-deoxy A is no longer acylable, so that the observed AMP–ATP exchange cannot be due to the reverse reaction. Sprinzl et al. [5] reported that this modified $tRNA^{Phe}$ is non-acylable. Fig.2 shows the time dependent acylation of $tRNA^{Phe}$, $tRNA^{Phe}$ 2′-deoxy A and $tRNA^{Phe}$ ox: slight residual acylation can be detected for $tRNA^{Phe}$ 2′-deoxy A as well as for $tRNA^{Phe}$ ox. For $tRNA^{Phe}$ 2′-deoxy A, this residual acylation cannot be attributed to some contamination by normal $tRNA^{Phe}$, since the modified $tRNA^{Phe}$ has been periodate oxidised to eliminate molecules which would have been recharged with normal adenosine at the 3′ terminus. But if the 2′-deoxy ATP used is somewhat contaminated by the 3′ isomer, this will result in the synthesis of $tRNA^{Phe}$ 3′-deoxy A, which cannot be destroyed by periodate oxidation and which is fully chargeable, as demonstrated by Sprinzl et al. [5]. The initial rate of residual acylation observed for $tRNA^{Phe}$ 2′-deoxy A only amounts to 0.4% of the initial rate of acylation found for normal $tRNA^{Phe}$ under the same conditions. The initial rate of the AMP–ATP exchange resulting from the overall reverse reaction should in fact be proportional to the concentration of Phe $tRNA^{Phe}$ produced. So, it should be linearly correlated to the initial rate of acylation. This is not the case, since the AMP–ATP exchange rate is around 5% of that of the reverse reaction in the presence of $tRNA^{Phe}$, whereas the residual acylation rate is only 0.4% with respect to normal $tRNA^{Phe}$. The observed AMP–ATP exchange must therefore be attributed to a mechanism other than the simple reverse reaction.

Clearly enough, the present result does not demonstrate the existence of a covalent acylenzyme intermediate. An alternative explanation could be, for instance, that upon tRNA binding some conformational change of the enzyme occurs, allowing an external AMP molecule to attack the aminoacyl-adenylate. But in any case, the observed AMP–ATP exchange triggered by the binding of tRNA leaves place for a mechanism involving an acylenzyme intermediate. A conformational transition of the protein upon tRNA binding can be correlated to the sedimentation coefficients observed for the free enzyme and the tRNA–enzyme complex [8]. The enzyme–tRNA complex exhibits a sedimentation constant of $S^0_{20,w}$ = 12S, whilst the enzyme alone sediments with a value of 8. 1S. The important increase of $S_{20,w}$ for the complex cannot be simply accounted for by an increase of 50 000 mol. wt. [8]. The enzyme molecule appears to shrink (or to become more globular) when it binds $tRNA^{Phe}$. Such a structural modification of the enzyme could be responsible for the observed modification of the catalytic properties of PRS. Experiments are under way which should allow the characterization of the covalent intermediate, if such a complex is involved in the catalytic mechanism.

Acknowledgements

We thank N. Menard for her technical assistance and M. Schlegel for the isolation of yeast $tRNA^{Phe}$. This work was partly supported by grants from the Delegation Generale à la Recherche Scientifique et Technique and from the Commissariat à l'Energie Atomique.

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