YEAST PHENYLALANYL–tRNA SYNTHETASE

Stimulation of the hydrolysis of enzyme bound aminoacyladenylate upon binding of tRNA

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1. Introduction

In a previous paper [1], we reported the triggering of an AMP–ATP exchange, catalysed by yeast phenylalanyl-tRNA synthetase (PRS), upon binding of $tRNA^{Phe}$. This modification of the catalytic properties of PRS upon binding of $tRNA^{Phe}$, together with the involvement of thiol groups in the acylation activity and the existence of an intermediate different from the aminoacyladenylate [2] led us to postulate the formation of an intermediate thioacylenzyme, triggered by the binding of $tRNA^{Phe}$ [1].

In this paper, we show that, according to the proposed mechanism, modified $tRNA^{Phe}$ molecules, such as $tRNA^{Phe}_{2'deoxy\ A}$ (in which the terminal adenosine has been replaced by 2'deoxyadenosine) and $tRNA^{Phe}_{ox}$ (in which the terminal adenosine has been periodic oxidised), stimulate the hydrolysis of enzyme bound aminoacyladenylate.

2. Materials and methods

The yeast phenylalanyl-tRNA synthetase was prepared by the procedure previously described [3]. Purified yeast $tRNA^{Phe}$ was obtained by countercurrent distribution, as described by Dirheimer and Ebel [4]. 2'Deoxy-ATP was obtained from Sigma (Missouri). 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) and the Radiochemical Center (Amersham, England). $tRNA^{Phe}_{ox}$ was prepared as previously described [1] and $tRNA^{Phe}_{2'deoxy\ A}$ was synthetized according to the method of Sprinzl et al. [5] as described in [1]. The phenylalanyl-adenylateenzyme complex was prepared according to Fasiolo et al. [6]. The reaction mixture had the following composition: 80 mM Tris–HCl buffer, pH 7.2, 12 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 4 mM Phe, 200 μM [^{14}C]ATP (5 μCi) or 4 mM ATP, 200 μM [^{14}C]Phe (5 μCi), 10 units pyrophosphatase and 300 μg PRS. The mixture (final vol. 0.25 ml) was incubated for 3 min at 37°C and then filtered at 4°C on a Sephadex G-25 column (0.8 × 23 cm) equilibrated with 50 mM succinate buffer, pH 6.0, 50 mM KCl, 1 mM EDTA, 5 mM 2-mercaptoethanol. The adenylate enzyme complex was usually recovered in 0.9–1 ml. The stoichiometry of the complex ranged between 1 and 1.5. Half of the fraction was mixed with the modified tRNA solution (1 nmol $tRNA^{Phe}$ 2'deoxy A in 10 μl or 4 μmol of $tRNA^{Phe}_{ox}$ in 60 μl), the other half being mixed with water instead of the tRNA solution. The mixtures were incubated at 25°C. At given time intervals, 50 μl aliquots were withdrawn and filtered onto nitrocellulose membranes (0.22 μM) which had previously been soaked for two hours in 50 mM succinate buffer, pH 6.0. The filters were then rinsed twice with 1 ml buffer, dried and counted in a scintillation counter.

For the experiment performed with an organomercurial modified enzyme, PRS was previously treated for 1 min at 0°C with a 20-fold molar excess of *p*-aminophenylmercuriacetate, as described in [2]. The reaction was stopped by addition of a stoichiometric amount of 2-mercaptoethanol. The modified enzyme had a residual activating capacity of 91% and a residual acylation activity of 46%.

3. Results and discussion

Figure 1 (a,b) show the hydrolysis of enzyme-bound aminoacyladenylate, in the absence or presence of modified $tRNA^{Phe}$ and without added Mg^{2+}. As can be seen, both tRNAs strongly stimulate the hydrolysis of the Phe ~ AMP–PRS complex. Table 1 summarises the results obtained in several experiments. $tRNA^{Phe}_{2'deoxy\ A}$ appears to be less efficient than $tRNA^{Phe}_{ox}$, but this effect may be due to the lower concentration of the former tRNA in the experiment. From table 1 it also appears that the release of AMP is faster than that of Phe. This difference was observed in all the experiments and demonstrates that this release of AMP and Phe corresponds to the hydrolysis of enzyme-bound aminoacyladenylate and not to a breakdown of free aminoacyladenylate. This slower release of Phe as compared to ATP is consistent with a possible transfer of the aminoacid to an accepting group of the protein, upon binding of $tRNA^{Phe}$. If such an intermediate is involved in the acylation of tRNA, it must be very reactive and therefore unstable enough to allow the transfer of the activated amino-acid to the solvent in the absence of an acylable tRNA.

Table 1
Half-life (in min) of the aminoacyl-adenylate enzyme complex labelled with [^{14}C]Phe or with [^{14}C]AMP

	without $tRNA^{PHE}$		with $tRNA^{PHE}$		Stimulation	
	^{14}C PHE	^{14}C AMP	^{14}C PHE	^{14}C AMP	^{14}C PHE	^{14}C AMP
exp. I	90	74	30	24	3,0	3,1
exp. II	80	66	34	28	2,3	2,4
exp. III	93	81	63	55	1,5	1,5

Stimulation of hydrolysis by $tRNA^{Phe}_{ox}$ (exp. I, exp. II) and $tRNA^{Phe}_{2'deoxy\ A}$ (exp. III).

It must be emphasized that some thiol groups of the protein appear to be important in the hydrolysis of enzyme-bound adenylate. This is illustrated in fig.2 (a,b): the aminoacyladenylate–PRS complex was formed with an enzyme previously labelled with *p*-aminophenylmercuriacetate under mild conditions (see Materials and methods and ref. [2]). This treatment gave rise to a modified enzyme, which was fully active in the PP_i–ATP exchange reaction (91%

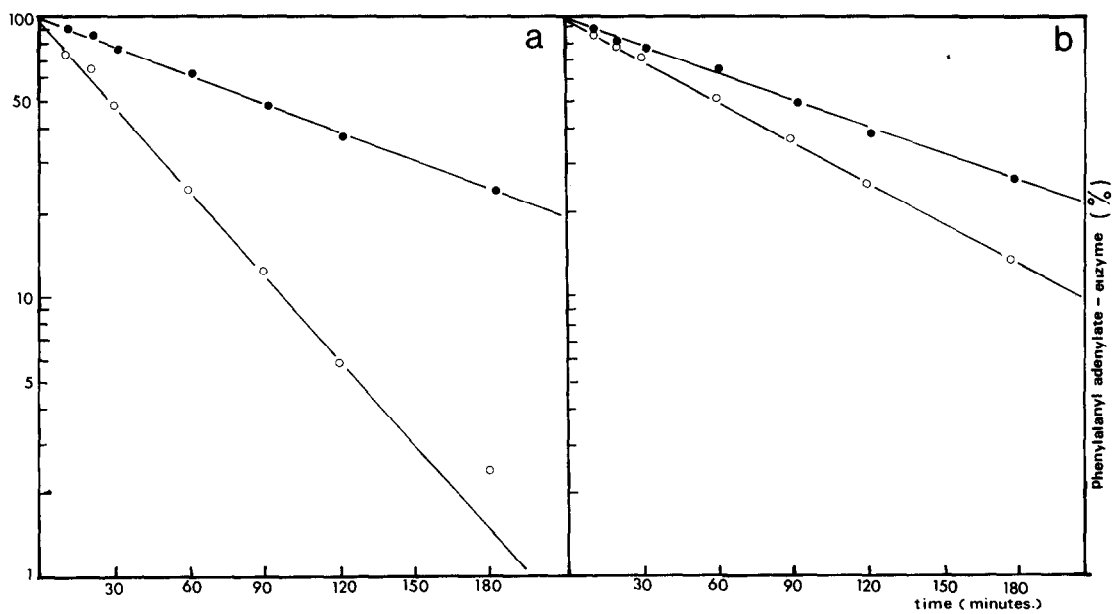

Fig.1. Stimulation by modified $tRNA^{Phe}$ of the hydrolysis at 25°C of [^{14}C]Phe-AMP–enzyme complex: $tRNA^{Phe}_{ox}$ (a) or $tRNA^{Phe}_{2'deoxy\ A}$ (b). (•–•) Control without modified $tRNA^{Phe}$. (○–○) With modified $tRNA^{Phe}$.

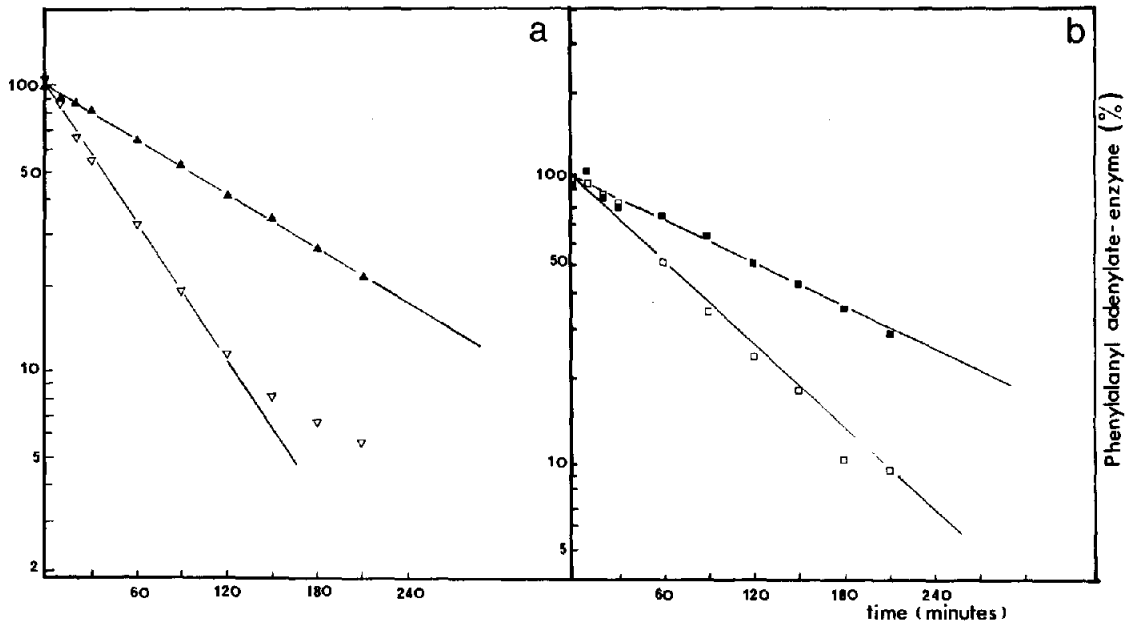

Fig.2. Effect of -SH groups modification on the stimulation by $tRNA^{Phe}_{ox}$ of the hydrolysis of [^{14}C]Phe-AMP–enzyme complex. (a) Non-treated enzyme: (▼–▼) complex alone, (△–△) complex + $tRNA^{Phe}_{ox}$; (b) PRS previously treated by *p*-aminophenylmercuriacetate (see text): (■–■) complex alone, (□–□) complex + $tRNA^{Phe}_{ox}$.

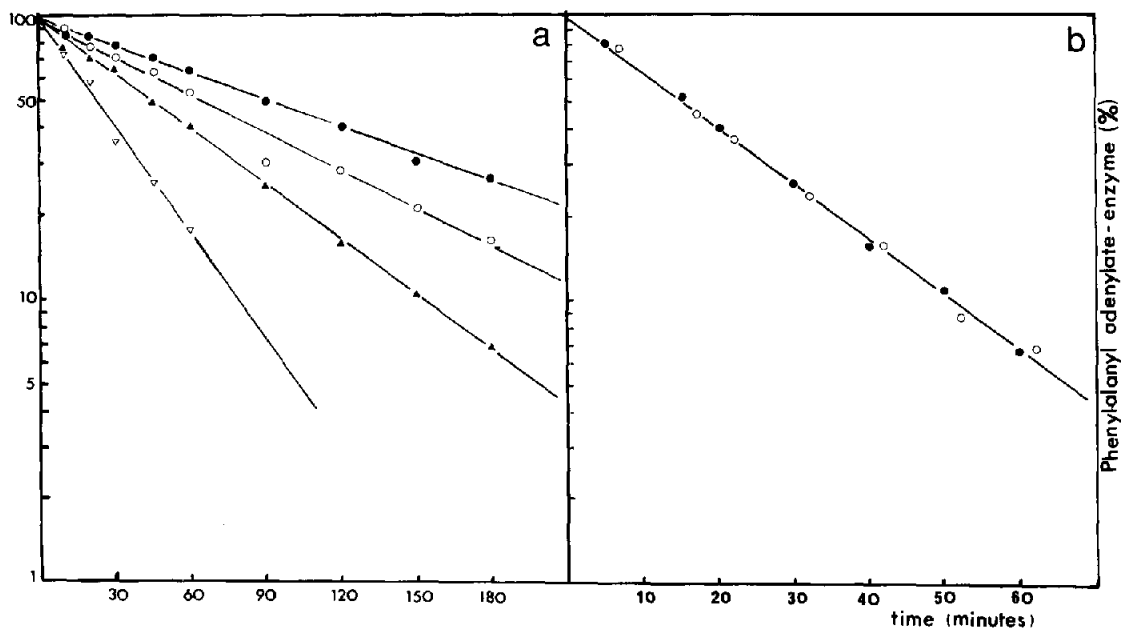

Fig.3. (a) Effect of different concentrations of $MgCl_2$ on the stability of the [^{14}C]Phe-AMP–enzyme complex at 25°C. (●–●) $MgCl_2$ (10^{-4} M), (○–○) $MgCl_2$ (10^{-3} M), (▼–▼) $MgCl_2$ ($2 \cdot 10^{-3}$ M), (▽–▽) $MgCl_2$ ($5 \cdot 10^{-3}$ M). (b) Hydrolysis of the [^{14}C]Phe-AMP–enzyme complex at 25°C in presence of 10 mM $MgCl_2$, without $tRNA^{Phe}_{ox}$ (●–●) or with $tRNA^{Phe}_{ox}$ (○–○).

of residual activity), but which had lost 54% of its acylation activity. This modification resulted in a more stable adenylate–enzyme complex, since its half-life was increased to 115 min in the absence of $tRNA^{Phe}$. Simultaneously, the stimulation of the hydrolysis upon binding of $tRNA^{Phe}_{ox}$ was less efficient, since for the control enzyme, the stimulation factor was 2.7, only 1.9 for the modified enzyme. This result is consistent with a possible participation of cysteinyl residues, which have been shown to be necessary for the aminoacylation activity of PRS, in the attack of the adenylate, formation of a thioacyl-enzyme and transfer of the amino acid to the tRNA (Murayama et al. [2]). The presence of the tRNA in its specific binding site seems to be required for the formation of the assumed intermediate, since we previously demonstrated that $tRNA^{Phe}$ was necessary for an AMP–ATP exchange to proceed (Remy and Ebel [1]) and since it also destabilises the adenylate–enzyme complex.

All the preceding experiments were performed in the absence of Mg^{2+}, since it has been shown that this cation is only required for the activation of the amino acid, but not for its transfer to the tRNA (Fasiolo et al. [6]). The results shown in fig.3 were thus unexpected. Figure 3a shows the hydrolysis of enzyme-bound adenylate for different Mg^{2+} concentrations. A strong stimulation of the hydrolysis was observed when Mg^{2+} concentration was increased from 10^{-4}– $5 \cdot 10^{-3}$ M. This result raises the question as to whether the stimulation observed in the presence of $tRNA^{Phe}_{ox}$ is due to Mg^{2+} ions which could contaminate the tRNA solution. But it must be noticed that the Mg^{2+} concentration required to bring the half-life of the adenylate–enzyme complex to 30 min (value observed in the presence of $tRNA^{Phe}_{ox}$) is between $2 \cdot 10^{-3}$ M and $5 \cdot 10^{-3}$ M. Since the highest concentration of tRNA used in the experiment is $8 \cdot 10^{-6}$ M, the minimum Mg^{2+} contamination should be 250 Mg^{2+} ions/tRNA molecule, which can absolutely be ruled out.

As can be seen on fig.3b, in the presence of 10 mM Mg^{2+} the stimulation of the adenylate hydrolysis by $tRNA^{Phe}_{ox}$ can no longer be detected. This could mean that Mg^{2+} and tRNA have the same effect on PRS structure and activity. Indeed, salts were reported to promote the interconversion between two forms of PRS differing by their catalytic properties (Von der Haar [7]). However, we do not favour this hypothesis: indeed if Mg^{2+} ions stimulate the transfer of the aminoacyl moiety to a side-chain of the protein, an AMP–ATP exchange should occur. We previously showed that this exchange did not occur in the absence of tRNA, even in the presence of 10 mM $MgCl_2$.

4. Conclusion

Although they do not demonstrate the existence of a thioacylenzyme in the reaction catalysed by yeast PRS, all the results reported hereby are consistent with the involvement of such an intermediate. Our previous results [1] which showed the requirement of $tRNA^{Phe}$ for the triggering of an AMP–ATP exchange (which has to be observed if an intermediate acyl-enzyme is involved in the reaction), are further supported by the observation that the adenylate–enzyme complex is strongly destabilised upon binding of tRNA. Furthermore, the integrity of PRS thiol groups appears to be an important factor for this destabilisation.

Acknowledgements

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References

[1] Remy, P. and Ebel, J. P. (1976) FEBS Lett. 61, 28–31.
[2] Murayama, A., Raffin, J. P., Remy, P. and Ebel, J. P. (1975) FEBS Lett. 53, 15–22.
[3] Fasiolo, F. and Ebel, J. P. (1974) Eur. J. Biochem. 49, 257–263.
[4] Dirheimer, G. and Ebel, J. P. (1967) Bull. Soc. Chim. Biol. 49, 1679–1687.
[5] Sprinzl, M., Scheit, K. H., Sternbach, H., Von der Haar, F. and Cramer, F. (1973) Biochim. Biophys. Res. Commun. 51, 881–887.
[6] Fasiolo, F. and Ebel, J. P. (1974) Eur. J. Biochem. 49, 257–263.
[7] Von der Haar, F. (1976) Eur. J. Biochem. 64, 395–398.