THE MECHANISM OF SALT-INDUCED STIMULATION OF $tRNA^{Ser}$ AMINOACYLATION BY YEAST SERYL-tRNA SYNTHETASE

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1. Introduction

The aminoacylation of tRNA is sensitive to changes in ionic strength [1]. For several aminoacyl-tRNA synthetases a salt-induced stimulation of the rate of tRNA esterification has been observed at moderate salt concentrations (e.g., [2–4]) but also a strong inhibition by different salts already at 25 mM was reported [5]. High concentrations of salt generally seem to inhibit the aminoacylation reaction, the only exception known being the esterification of tRNA from halophilic organisms [6]. Extremely high salt concentrations also stimulate the aminoacylation of yeast $tRNA^{Phe}$ by the phenylalanyl-tRNA synthetase from *Escherichia coli* [7], whereas generally the aminoacylation of non-cognate tRNAs is favored by low ionic strength [8]. Various explanations of the different salt effects were proposed, among them a salt-dependent conformational change of the synthetase [4], stabilization of a certain tRNA conformation [2,9], or an interference by salt with the interaction between synthetase and tRNA [1,10].

With seryl-tRNA synthetase (SRS) from yeast it was found that the rate of $tRNA^{Ser}$ aminoacylation increases with increasing concentrations of KCl [11, 12]. On the basis of relaxation kinetic studies on $tRNA^{Ser}$ dissociation from SRS [11] the stimulating effect of KCl was explained by a salt-induced acceleration of seryl-$tRNA^{Ser}$ dissociation [12]. However, recent investigations [13] revealed that SRS differs from other aminoacyl-tRNA synthetases in that the activation of serine is the rate limiting step of $tRNA^{Ser}$ aminoacylation and not the dissociation of the seryl-$tRNA^{Ser}$–SRS complex. We therefore have examined the question further and found the serine activation to occur much faster in the absence than in the presence of $tRNA^{Ser}$. This allows the conclusion that salt stimulates the aminoacylation of $tRNA^{Ser}$ at its rate limiting step, i.e., at the activation reaction, by partially dissociating the SRS–$tRNA^{Ser}$ complex. Such a mechanism may also apply to other aminoacyl-tRNA synthetase systems.

2. Materials and methods

SRS and $tRNA^{Ser}$ from yeast were prepared as in [12,14]. Yeast $tRNA^{Phe}$ was a gift of R. Thiebe and W. Wintermeyer. Inorganic salts (p.a.) were obtained from Merck (Darmstadt); other materials were as in [13].

All experiments were performed at 25°C in the pH 7.0 buffer system described in [13]; upon addition of salt the pH-value was readjusted if necessary. Substrate concentrations employed were saturating, at least in the absence of salt. Aminoacylation experiments were conducted according to [15]. Hydrolysis of [γ-^{32}P]ATP and $^{32}PP_i$–ATP exchange were measured similarly as in [16].

3. Results and discussion

When both the concentrations of KCl and $MgCl_2$ were varied in aminoacylation experiments, the rate maximum was observed at 0.6 M KCl (fig.1) instead of 0.2 M KCl found in [11,12] at a fixed (relatively low) $MgCl_2$ concentration. With increasing concentrations of KCl the optimal $MgCl_2$ concentration also increases. Probably K^+ compete with Mg^{2+} for sites in catalytically important ATP, $tRNA^{Ser}$ or possibly synthetase complexes. Only if this competition is balanced by

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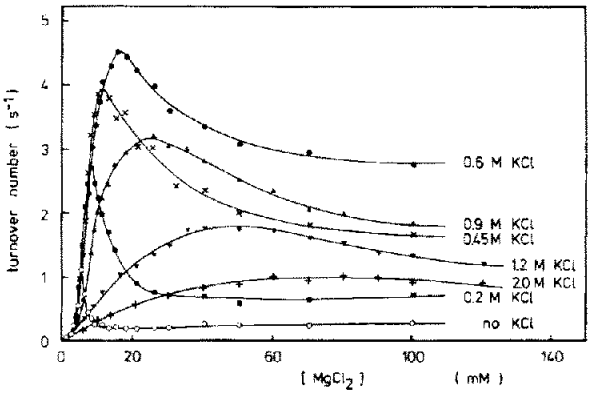

Fig.1. Influence of KCl and $MgCl_2$ concentrations on the steady state rate of $tRNA^{Ser}$ aminoacylation. The reaction mixture contained: 2 nM SRS, 5 mM ATP, 0.5 mM [^{14}C]-serine, 20 μM $tRNA^{Ser}$, $MgCl_2$ and KCl as indicated.

increased concentrations of $MgCl_2$, is the maximum aminoacylation rate achieved. In an ancillary study the influence of various salts on the rate of $tRNA^{Ser}$ esterification was compared (fig.2). While the special effects of $(NH_4)_2SO_4$ and KSCN are not explained, the order of salt dependent stimulation by KNO_3 > KJ > KBr > KCl > KOAc parallels the one of the chaotropic effect of the anions.

Clues as to the mechanism of salt-induced stimulation of SRS activity were obtained from two types of reactions which can be run in the absence and presence of $tRNA^{Ser}$; this is $^{32}PP_i$–ATP exchange and [γ-^{32}P]-ATP hydrolysis. The rate of PP_i–ATP exchange at

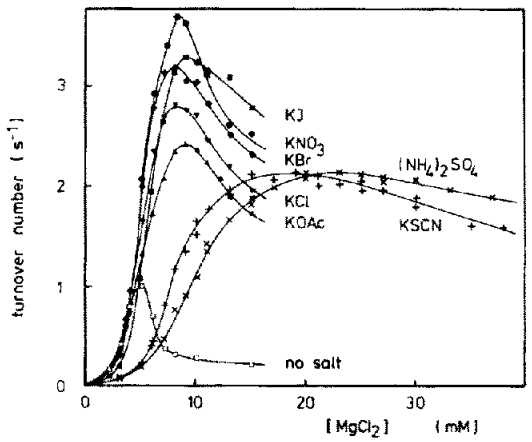

Fig.2. Comparison between the effects of equimolar concentrations (0.2 M) of different salts on the steady state rate of $tRNA^{Ser}$ aminoacylation and its dependence on $MgCl_2$. Substrate concentrations were as given in fig.1.

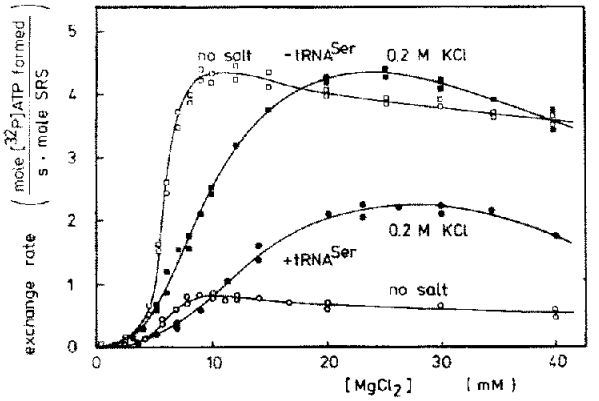

Fig.3. Effects of $tRNA^{Ser}$ (20 μM) (○,●) and KCl (0.2 M) (●,■) on the $^{32}PP_i$–ATP exchange rate and its dependence on $MgCl_2$. Substrate concentrations were: ATP 5 mM, $^{32}PP_i$ 2 mM, serine 0.5 mM, $MgCl_2$ as indicated. It should be noted that the rate maxima are not at the same $MgCl_2$ concentrations as in fig.1,2; this is due to the presence of PP_i in the exchange experiments.

optimal $MgCl_2$ was about the same independent of whether KCl was present or not when the reactions were carried out in the absence of $tRNA^{Ser}$ (fig.3). Also other salts had no stimulating effect (not shown). In the presence of $tRNA^{Ser}$, however, the exchange rate is lower by a factor of 7 (fig.3) than in the absence of $tRNA^{Ser}$. The rate is stimulated in the presence of

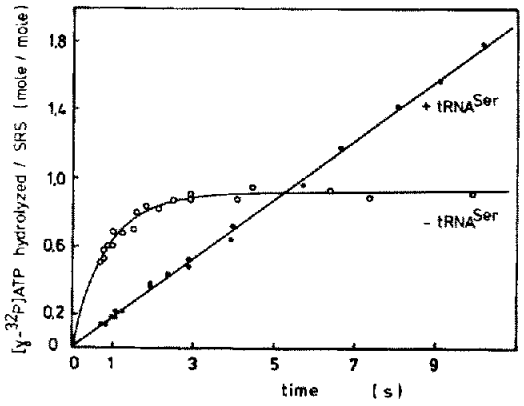

Fig.4. Time curves of serine-dependent hydrolysis of [γ-^{32}P]-ATP in the absence (○) and presence (●) of $tRNA^{Ser}$. The reaction mixture contained 3 μM SRS, 160 μM [γ-^{32}P]ATP, 0.5 mM serine, 1.5 mM $MgCl_2$, and 20 μM $tRNA^{Ser}$ if present. Blank values as measured in the absence of serine were subtraced. Evaluation of the curves yielded the initial rate of serine activation in the absence of $tRNA^{Ser}$ and the steady state rate of ATP hydrolysis in the presence of $tRNA^{Ser}$ which is practically equal to the rate of serine activation under this condition [13].

KCl: at 0.2 m KCl it is lower only by a factor of 2 in the presence of $tRNA^{Ser}$ as compared to the exchange rate measured in the absence of $tRNA^{Ser}$ (fig.3); at 0.6 M and 1.2 M KCl the two rates differ by ~15% and 10%, respectively (not shown). When $tRNA^{Phe}$ was added instead of $tRNA^{Ser}$ no effect was observed (not shown). Upon addition of $tRNA^{Ser}$ also the rate of serine activation as measured by [γ-^{32}P]ATP hydrolysis decreased by a factor of 7 (from 1.2–0.17 s^{-1}) as can be derived from the curves of fig.4.

In fig.1 a systematic study of the salt dependence of the rate of seryl-$tRNA^{Ser}$ formation is described. The decrease of the aminoacylation velocity in the high salt range is certainly related to the low concentration of the SRS–$tRNA^{Ser}$ complex at those salt concentrations. The stimulation by salt of the rate of serine activation in the low and intermediate concentration range is explained, on the basis of fig.3,4, by the observation that the reaction proceeds faster in the absence than in the presence of $tRNA^{Ser}$ as was also observed in other aminoacyl-tRNA synthetase systems (e.g., [17,18]). With increasing salt concentrations the dissociation of the SRS–$tRNA^{Ser}$ complex is favored and the concentration of the free SRS, which is more efficient in the activation reaction, is higher. This interpretation is in agreement with the results of relaxation kinetic studies on the influence of salt on the rates of $tRNA^{Ser}$ binding and dissociation [11] and with the results of stopped flow measurements on the association of $tRNA^{Ser}$ and seryl-$tRNA^{Ser}$ with SRS [19]. The stimulation of the aminoacylation reaction by salt is therefore not due to a direct influence of the salt on the activation step but rather to a salt-induced partial dissociation of the SRS–$tRNA^{Ser}$ complex. Modulation of the rate of amino acid activation by such a mechanism may be operating also in other aminoacyl-tRNA synthetases irrespective of whether or not in these systems the activation is the rate limiting step of the aminoacylation reaction.

Acknowledgements

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