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Stimulatory effect of ammonium sulfate at high concentrations on the aminoacylation of tRNA and tRNA-like molecules

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The influence of various salts on the aminoacylation of $tRNA^{Val}$ and the tRNA-like structure from turnip yellow mosaic virus RNA by yeast valyl-tRNA synthetase has been studied. As expected, increasing the concentration of salts inhibits the enzymatic reaction. However, in the presence of high concentration of ammonium sulfate, and only this salt, the inhibitory effect is suppressed. Under such conditions, the aminoacylation becomes comparable to that measured in the absence of salt. It was shown that ammonium sulfate affects both the catalytic rate of the reaction and the affinity between valyl-tRNA synthetase and the RNAs. Because the affinity between the partners in the complex is increased when the concentration of the salt is high, it is suggested that hydrophobic effects are involved in tRNA/synthetase interactions.

Valyl-tRNA synthetase; $tRNA^{Val}$; tRNA-like; Ammonium sulfate; Aminoacylation

1. INTRODUCTION

Many types of forces contribute to the binding energy and specificity of nucleic acid/protein interaction [1–3]. Since nucleic acids are polyelectrolytes, a well accepted assumption is that electrostatic forces play the predominant role. Consequently, salts should inhibit nucleoprotein complex formation [2]. In the field of aminoacyl-tRNA synthetase recognition of tRNA it is well known that salts, such as sodium chloride, behave in such a way (e.g. [4–7]). Unexpectedly however, it has been found that the complex between yeast aspartyl-tRNA synthetase and $tRNA^{Asp}$ can be crystallized in the presence of ammonium sulfate at high concentration [8–10]. Interestingly, in such conditions the enzymatic activity is only slightly decreased [11]. This effect of ammonium sulfate is not restricted to the aspartic acid system. A specific stabilization by this salt was also observed in the case of the ternary complex EF-Tu/GTP/valyl-$tRNA^{Val}$ [12]. More recently, high concentration of ammonium sulfate allowed the crystallization of the complex between *Escherichia coli* $tRNA^{Gln}$ and glutaminyl-tRNA synthetase [13]. All these data suggest that the observed effects are due to peculiar physicochemical properties of ammonium sulfate such as its salting out behavior [1], which likely favor formation of hydrophobic interactions between proteins and RNAs.

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To better understand the salt effects and to generalize the above assumptions, the influence of various salts on the interaction of two RNAs with yeast valyl-tRNA synthetase was studied. It was shown that complex formation and valylation of the tRNA-like structure of turnip yellow mosaic virus (TYMV) RNA and of $tRNA^{Val}$ occurs at high concentration of ammonium sulfate in contrast to what is observed with other salts, which exhibit inhibitory effects. These observations will be discussed in light of the existence of a balance between electrostatic and hydrophobic forces involved in protein/RNA complexes, which is shifted towards the hydrophobic component in the presence of ammonium sulfate.

2. MATERIALS AND METHODS

2.1. *Macromolecules and chemicals*

Valyl-tRNA synthetase (a monomer of M_r 130000) and $tRNA^{Val}$ (M_r 25000) were purified from *Saccharomyces cerevisiae* as previously described [14,15]. The enzyme exhibts a specific activity of 3000 units per mg (nmol of valine $\cdot mg^{-1} \cdot min^{-1}$); its concentration was calculated from an extinction coefficient at 280 nm of 1.79 $cm^2 \cdot mg^{-1}$. A tRNA-like fragment (159 nucleotides) was purified from TYMV RNA as described in [16]. Concentrations of the tRNA-like molecule and of $tRNA^{Val}$ were calculated assuming that 1 absorbance unit at 260 nm corresponds to 39 and 35 $\mu g \cdot ml^{-1}$, respectively. tRNA-nucleotidyl transferase was used to reconstruct the CCA terminus prior to valylation of the tRNA-like molecules [16]. L-[^{3}H]Valine (25 Ci/mmol) was from the Commissariat à l'Energie Atomique (Saclay, France). Ammonium sulfate, enzyme grade, was from BRL (Gaithersburg, MD, USA).

2.2. *Aminoacylation assays*

Aminoacylation assays were performed under optimized condi-

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tions derived from [14]. Incubation media (50 μl) contained 100 mM Tris-HCl at pH 8.0, 30 mM KCl, 12.5 mM $MgCl_2$, 5 mM ATP, 2.5 mM glutathione, 80 μM tritiated valine and the required salt. The concentrations of $tRNA^{Val}$ or tRNA-like fragment were 300 to 800 μM. Reactions were initiated by addition of a catalytic amount (100 nM) of valyl-tRNA synthetase. Aliquots of 20 μl were withdrawn after 4 and 8 min incubation at 37°C and deposited on Whatman 3MM paper discs. The radioactive valyl-tRNA was counted in a liquid scintillation counter [14].

2.3. *Nitrocellulose disc filtration*

Tritiated valyl-$tRNA^{Val}$, aminoacylated in the conventional manner, was used for affinity measurements with valyl-tRNA synthetase. Affinity constants of tRNA for the synthetase were determined by titration experiments using a nitrocellulose membrane filtration method [17] as modified in [6,7]. The medium was 100 mM potassium phosphate buffer at pH 6.0, 2 mM $MgCl_2$, 30 mM KCl, 400 mM NaCl, supplemented if needed with ammonium sulfate (4 M stock solution adjusted at pH 7.0). Concentration of tRNA varied from 0.1 to 0.35 mM and that of the synthetase was 1 mM. Before use nitrocellulose filters (Sartorius) were washed in the phosphate buffer supplemented with bovine serum albumin 10 $mg \cdot l^{-1}$. The retention yield of the filters was 15–25%. Affinities were evaluated from a Scatchard analysis. For one set of measurements 3 mg of tRNA and 4 mg of pure synthetase were needed.

Table 1

Percentage aminoacylation of yeast $tRNA^{Val}$ by yeast valyl-tRNA synthetase in the presence of various salts

Salt	Ionic strength									
	0	0.4	0.8	1.2	2.2	2.7	3.2	3.4	3.6	4.0
NaCl	100	6.5	0.2	0	0	–	0	–	–	–
$(NH_4)_2SO_4$	100	42	24.5	22	15	–	–	–	–	–
$(NH_4)_2SO_4$ + NaCl (400 mM)	–	6.5	–	0.5	0.8	1.6	–	4	–	4
NH_4Cl	100	67	2.0	0.3	0	–	0	–	–	–
NH_4Cl + NaCl (400 mM)	–	6.5	1.3	0.4	0	0	–	–	0	–
Na_2SO_4	100	4	0	0	0	–	0	–	–	–
Na_2SO_4 + NaCl (400 mM)	–	6.5	0.6	0	0	–	–	0	–	–
KSCN	100	0.5	0	0	0	–	0	–	–	–
KSCN + NaCl (400 mM)	–	6.5	1.8	0	0	–	–	0	–	–

3. RESULTS AND DISCUSSION

3.1. *Valylation of $tRNA^{Val}$ and of a tRNA-like molecule as a function of ammonium sulfate*

Fig.1A shows the variation of the valylation rate of yeast $tRNA^{Val}$ as a function of ammonium sulfate concentration. As was observed with the aspartic acid system from yeast [11], the enzymatic rate decreases monotonically when the salt concentration increases, but remains significant (15%) at concentrations as high as 1.6 M. This contrasts with what is observed in NaCl, which at 400 mM exhibits a strong inhibitory effect on valylation (6.5% remaining activity) (table 1). A likely explanation for the ammonium sulfate effect at high concentrations would be the establishment of salt induced hydrophobic forces stabilizing the nucleoprotein complex. The fact that the aspartyl-tRNA synthetase/$tRNA^{Asp}$ complex crystallizes with ammonium sulfate [8–10] is a good argument in favor of this view.

Because electrostatic interactions contribute to the stabilization of the complexes at low ionic strength [6,7], but also because ammonium sulfate favors hydrophobic effects [18], the aminoacylation curve displayed in fig.1A should reflect a combination of both electrostatic and hydrophobic effects. Since electrostatic interactions are strongly diminished at rather low ionic strength (see the NaCl effect) one expects an ammonium sulfate curve with a trough: first a decrease of the rate, reflecting the progressive loss of electrostatic interactions followed by an increase of rate corresponding to the establishment of hydrophobic interactions (at very high salt concentrations, the activity again should decrease, because of the salting out of the macromolecules). This predicted behavior is not observed for the aminoacylation of $tRNA^{Val}$ (fig.1A), nor for that of $tRNA^{Asp}$ [11]. Under optimal aminoacylation conditions, the interaction between the tRNA and the synthetase may be too strong so that the hydrophobic interactions at high ammonium sulfate concentration cannot completely overcome the loss of electrostatic effects. As a consequence, the predicted biphasic curve should appear when ligands with less affinity to valyl-tRNA synthetase are used. Such ligands, structural analogs to canonical tRNA, are found in viral tRNA-like fragments.

RNAs from several plant viruses can be aminoacylated at their 3′ end [19]. Although the sequences of these molecules differ markedly from those of tRNAs, they present three-dimensional features analogous to those found in canonical tRNA ([19–22] and references therein). The tRNA-like structure from TYMV is known to be efficiently aminoacylated by yeast valyl-tRNA synthetase [19]. Although the kinetic parameters of its valylation reaction are close to those found for $tRNA^{Val}$, an increase in K_M was observed, suggesting that the viral molecule interacts less strongly with valyl-tRNA synthetase than does $tRNA^{Val}$ [23]. As predicted the valylation of TYMV RNA as a function of ammonium sulfate concentration gives a biphasic curve with a trough in the presence of medium salt concentrations (fig.1B).

If the salt dependence observed with the tRNA-like molecule is only a consequence of the peculiar properties of ammonium sulfate, one should be able to define experimental conditions under which this effect can also be detected with canonical tRNA, the classical substrate of aminoacyl-tRNA synthetases. A way to do this is to further lower the strength of the electrostatic interactions in the system. This was done by supplementing the valylation medium by 400 mM NaCl. The experiment displayed in fig.1C actually shows a biphasic variation of $tRNA^{Val}$ aminoacylation rates as

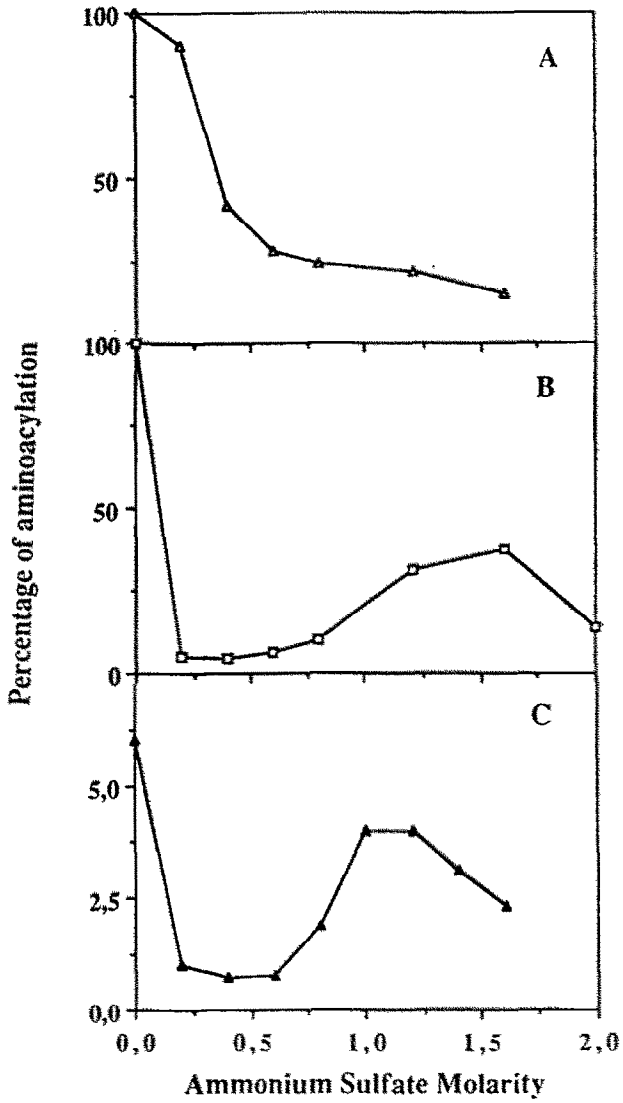

Fig.1. Initial rates of the aminoacylation of yeast $tRNA^{Val}$ (A and C) and of TYMV tRNA-like fragment (B) by yeast valyl-tRNA synthetase, as a function of ammonium sulfate concentration. The curve displayed in panel C was obtained by supplementing the aminoacylation medium with 400 mM NaCl.

a function of ammonium sulfate concentration, similar to what is observed with the tRNA-like structure of TYMV in the absence of NaCl. Note, however, that the maximum rate of aminoacylation of $tRNA^{Val}$ observed at high ammonium sulfate concentration and in the presence of NaCl does not exceed the values found under similar conditions in the absence of NaCl (compare figs 1A and C).

3.2. *Comparison of ammonium sulfate with other salts*

As discussed above, the effects of ammonium sulfate on aminoacylation rates differ from those observed with NaCl. Either ion, NH_4^+, SO_4^{2-}, or both ions, could be responsible for the observed effects; therefore we have tested the influence of ammonium chloride and sodium sulfate on $tRNA^{Val}$ aminoacylation. Table 1 clearly shows that high concentrations of these salts, either alone or supplemented with 400 mM NaCl, do not stimulate the enzymatic reaction rates. It can thus be concluded that the recovery of enzymatic activaties in the presence of ammonium sulfate is a consequence of the specific properties of the combination of ammonium and sulfate ions. In control experiments conducted in the presence of KSCN, a chaotropic salt destabilizing water shells around macromolecular structures [18,24] enzymatic activities were completely abolished, even at low concentrations of KSCN.

3.3. *Effect of ammonium sulfate on the affinities between tRNAs and valyl-tRNA synthetase*

In the above discussion, we made the implicit assumption that aminoacylation rates in the presence of salts are directly correlated to the strength of tRNA/aminoacyl-tRNA synthetase interactions and do not reflect possible modulations in enzymatic mechanisms. This last possibility has to be considered since tRNA aminoacylation, and in particular valylation [25], are multistep processes with rate limiting steps that could be different at low and high ammonium sulfate concentrations. If so, variations in valylation rates would not necessarily reflect variation in the strength of interaction between tRNA and synthetase. For example, a seryl-tRNA synthetase exhibits modulation by KCl of the rate limiting step of tRNA charging [26]. Therefore, to support the interpretation discussed above, it is important to check if the affinity between tRNA and synthetase is affected. This was first done by determination of Michaelis-Menten constants (fig.2). In agreement with our views, the inverse of K_M values, good approximations of affinities, first decrease with increasing ammonium sulfate molarities (0 to 0.3 M); further increasing the salt concentration leads to an increase of affinity. Represented in this way, the K_M dependence of $tRNA^{Val}$ with valyl-tRNA synthetase is superimposable on the initial rate dependence of valylation as shown in figs 1B and C. Noteworthy, V_{max} is affected by the presence of the salt and has an unexplained maximum at 0.3 M ammonium sulfate; moreover the specificity constant (V_{max}/K_M) exhibits at medium salt concentration, a minimum which could be due to both weak electrostatic and hydrophobic interactions. Affinity constants measured independently at two ammonium sulfate concentrations varied in the expected way: K_A at 300 mM ammonium sulfate is about $20 \cdot 10^3$ M^{-1}; at 700 mM of this salt it is increased by a factor of 5. It must, however, be emphasized that these numerical values are only approximate since measurements were made at the limit of feasibility (due to the low affinity caused by the presence of 400 mM NaCl and of unknown effects of high salt concentrations on filtration); however, we believe that their comparison is significant.

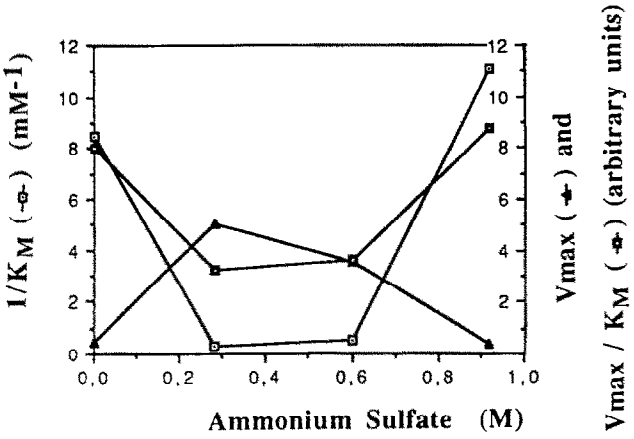

Fig.2. Variations of the kinetic constants, K_M and V_{max}, and of the specificity constant V_{max}/K_M of the aminoacylation of $tRNA^{Val}$ by valyl-tRNA synthetase as a function of ammonium sulfate concentration in the presence of 400 mM NaCl.

4. CONCLUSIONS

The present valylation studies of a tRNA-like structure and of $tRNA^{Val}$ show that complex formation between these RNAs and valyl-tRNA synthetase can be modulated by ammonium sulfate. Whereas low concentrations of this salt decrease the strength of the interactions, high concentrations in contrast allow stable and active complex formation. These data on the valine system extend earlier observations on other tRNA/protein complexes [11–13] and show that strong electrostatic interactions are not indispensable for interaction. Indeed they can be compensated by hydrophobic components as also proposed for DNA/protein complexes [3].

The complexes, stabilized by hydrophobic forces, remain catalytically active with specificity constants similar to those observed in the absence of ammonium sulfate (fig.2). However, the question remains open whether discrimination of valyl-tRNA synthetase against non-cognate tRNA is maintained under such conditions where electrostatic forces are minimized. From another point of view and as a practical consequence we would like to emphasize again the usefulness of ammonium sulfate as a crystallization precipitant of specific RNA/protein complexes [8–10,13]. In future work it would be important to investigate whether the peculiarity of ammonium sulfate similarly affects DNA/protein complexes, which have not yet been crystallized in the presence of this salt (e.g. [2,27]).

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