QUANTITATIVE STUDY OF THE IONIC INTERACTIONS BETWEEN YEAST $tRNA^{Val}$ AND $tRNA^{Phe}$ AND THEIR COGNATE AMINOACYL-tRNA LIGASES

J. BONNET, M. RENAUD, J. P. RAFFIN and P. REMY
Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue Descartes, 67000 Strasbourg, France.

Received 27 February 1975

1. Introduction

A general characteristic of the interactions between the tRNAs and the aminoacyl-tRNA ligases is that they are lowered by ionic strength. One explanation is that ionic strength weakens ionic interactions existing between phosphate groups of the tRNA and positive charges of the enzyme.

A quantitative study based on this assumption was undertaken by Loftfield [1]. Using the Debye-Hückel law, he found 2 to 4 charges interacting with the same number of positive ones. Since the Debye-Hückel law is valid only for small ions, the validity of these conclusions is questionable.

A second explanation for the salt effect was proposed by Yarus [2] who postulated that the interactions between tRNA and enzyme required a conformational change and that this change was inhibited by salt.

We report here a study of the affinity of $tRNA^{Val}$ and $tRNA^{Phe}$ for their cognate aminoacyl-tRNA ligases as a function of ionic strength under conditions where conformational changes can be considered as negligeable. Our results agree with the model of interaction between nucleic acids and protein developed by Daune [3–5]. Our data are consistent with the existence of 5–6 phosphate groups interacting with the same number of positive charges on the enzyme. The participation of ionic interactions in the binding of the tRNA by its cognate aminoacyl-tRNA ligase would be about 40 to 60% of the total energy of interaction under usual conditions. This model is also sufficient to explain the increase in tRNA affinity for its aminoacyl-tRNA ligase in the presence of methanol without postulating any conformational change.

At last, we studied the behaviour of a three quarter $tRNA^{Phe}$. The three quarter $tRNA^{Phe}$ molecule's behaviour could be interpreted as the result of the loss of 2 ionic interaction sites.

2. Materials and methods

Yeast valyl-tRNA ligase was prepared in this laboratory by D. Kern (D. Kern, R. Giegé and J. P. Ebel, in preparation) according to a procedure derived from that described by Lagerkvist and Waldenström [6]. Yeast phenylalanyl-tRNA ligase was purified according to Fasiolo et al. [7]. Yeast-$tRNA^{Phe}$ and $tRNA^{Val}$ were purified by counter current distribution followed (for $tRNA^{Val}$) by chromatography on RPC5 [8] and BD-cellulose [9]. Splitting in the dihydrouracile loop of $tRNA^{Phe}$ was performed according to Krebs et al. [10]. The fragments were prepared by chromatography on RPC5 using 6 M urea at pH 3.0.

We used [^{3}H]Val-$tRNA^{Val}$ to measure the affinity of $tRNA^{Val}$. It was previously verified that the amino acid linked to the tRNA had no detectable influence upon the interaction between valyl-tRNA ligase and cognate or non cognate tRNAs (J. Bonnet and J. P Ebel, submitted to Eur. J. Biochem.). In other systems the affinities of tRNA and aminoacyl-tRNA were also found to be equal [11–15].

The affinity constants of valyl-$tRNA^{Val}$ for valyl-tRNA ligase were determined by titration experiments. The amount of bound Val-$tRNA^{Val}$ to valyl-tRNA ligase was determined by filtration through nitrocellulose membrane according to Yarus and Berg, [16].

North-Holland Publishing Company – Amsterdam

The medium was: 50 mM phosphate buffer, pH 6.0; 0.5 mM Mg^{++}, bovine serum albumin 10 μg/ml, 5 mM 2-mercaptoethanol and various amounts of NaCl. A given amount of enzyme (6 to 180 nM, according to the ionic strength) was titrated by increasing amounts of valyl-$tRNA^{Val}$ (2 to 3000 nM). The rate of aminoacylation of $tRNA^{Phe}$ by phenylalanyl-tRNA ligase was determined in the following medium: bovine serum albumin 100 μg/ml; 1 mM glutathione; 20 mM Tris–HCl pH 7.4; 6 mM ATP; 10 mM $MgCl_2$; 0.82 μM $tRNA^{Phe}$; phenylalanyl-tRNA ligase 0.5 μg/ml; 0.1 mM [^{14}C] phenylalanine. NaCl was added at varying concentrations. K_m values were determined from

$$v = \frac{V \quad [tRNA^{Phe}]}{K_m + \ [tRNA^{Phe}]}$$

We verified that V remained constant in the conditions used. To verify that K_m was sufficiently close to the reciprocal of the affinity constant, direct measurements of this affinity were made. The affinity of $tRNA^{Phe}$ for phenylalanyl-tRNA ligase was determined by fluorescence titration of Y base according to Fasiolo et al. [17] in the following medium; 10 mM Tris–HCl buffer pH 7.2; 10 mM $MgCl_2$ and increasing concentrations of sodium chloride. The $tRNA^{Phe}$ concentration was 0.5 μM and increasing amounts of enzyme were added. The experiments were conducted at 12°C.

The affinity of the 3/4 $tRNA^{Phe}$ was determined by competition experiments. Aminoacylations were performed as above but 5.7 μM 3/4 $tRNA^{Phe}$ were added to the medium.

3. Results and discussion

It has been shown that sodium and magnesium ions act in a very similar way upon the structure of the tRNA (18, 19, 20, 21 and references therein). But, to have an identical effect, concentrations of sodium 100 to 1000 times larger than those of magnesium are required. The major conformational changes are achieved (below 40°C) for concentrations lower than 2 M Na^+ or 1 mM Mg^{++} [13–15].

Interactions between valyl-$tRNA^{Val}$ and valyl-tRNA ligase were performed at 0°C in 0.5 mM magnesium and 60 to 210 mM monovalent cations. Interactions between $tRNA^{Phe}$ and phenylalanyl-tRNA ligase were performed in 6 mM or 10 mM free magnesium at 37°C or 12°C and between 250 to 650 mM monovalent cations. Under these conditions the ionic strength due to magnesium ions on one hand and conformational changes produced by variations of monovalent ions concentration on the other hand can reasonably be neglected.

Fig.1 shows that in the range of ionic strength indicated above, the affinities of $tRNA^{Val}$ and $tRNA^{Phe}$ for their cognate aminoacyl-tRNA ligase are drastically reduced. It can therefore be concluded that the salt inhibition of tRNA-aminoacyl–tRNA ligase interactions is essentially due to a change in ionic interactions and not to a conformation effect on the tRNA level. These results were interpreted quantitatively by assuming that the region implicated in the ionic interactions is locally a cylindrical polyelectrolyte. This is a reasonable assumption since the X-ray determined three-dimensional structure of $tRNA^{Phe}$ [22,23] shows the whole molecule to be composed essentially of perpendicular double helixes. Moreover, based on these assumptions, Schuber and Pinck [24] were able to quantitatively interpret the variations in the deacylation rate of yeast Val-$tRNA^{Val}$ as a function of the ionic strength. We used the model worked out by Daune of protein-nucleic acid interpretation which has been successfully applied to the interactions between DNA and some proteins [3–5]. According to this model, the apparent association constant between a nucleic acid and a protein can be written:

$$\log K = C + a \log(k/1 + kc)$$

where: c is the concentration of monovalent cations. k is their affinity for the polynucleotide chain at a given ionic strength, a is the number of phosphate groups implicated in the interactions between tRNA and positive charges located on the protein, C corresponds to the energy of non ionic interactions.

For the higher values of the ionic strength, the logarithm of the association constant of $tRNA^{Val}$ for valyl-tRNA ligase is a linear function of $\log(k/1 + kc)$ (fig.1). The fit is not as good for lower salt concentrations. This could be due to the fact that the model is no longer valid for ionic concentrations below 50 mM [4]. The slope (5.6) of the straight line is compatible with the existence of 5 to 6 sites of ionic

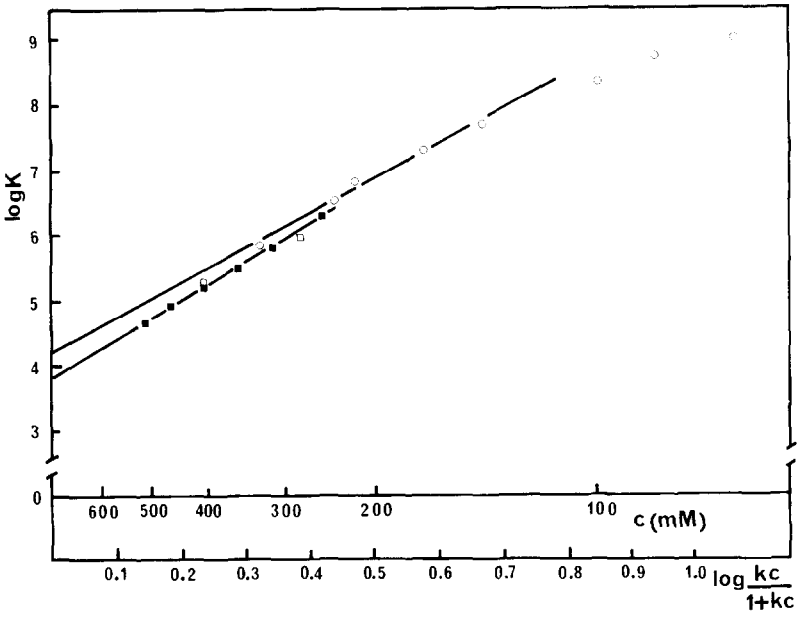

Fig.1. Effect of ionic strength on association of $tRNA^{Val}$ and valyl-tRNA ligase and $tRNA^{Phe}$ and phenylalanyl-tRNA ligase. The affinity of $tRNA^{Val}$, measured as that of Val-$tRNA^{Val}$ (○) and of $tRNA^{Phe}$ measured, in this last case either by fluorimetric titration (□) or as K_m^{-1} (■) were determined as described in materials and methods. The values of $\log(k/1 + kc)$ are drawn from [3] and [4].

interactions. The intercept of the straight line with the ordinate axis gives the value of C which is the value of $\log K$ for an infinite concentration of salt.

Thus the contribution of the ionic interactions to the total energy of interaction can be calculated under the conditions used. For the ionic strengths generally used (60 mM monovalent ion) to isolate the complex $tRNA^{Val}$–valyl-tRNA synthetase, $\log K$ was found equal to 9.2 (fig.1). Since non-ionic interactions correspond to a $\log K$ of 3.4, the ionic interactions contribute to 50% to the total energy of interaction.

The influence of ionic strength upon the association $tRNA^{Phe}$-phenylalanyl-tRNA ligase is in good agreement with the model at any salt concentration (fig.1). The slope of the straight line is 6.0 and the extrapolation indicates that under the usual aminoacylation conditions (about 150 mM of cations), the contribution of ionic interactions to the total energy of interaction is 40%.

Fig.2 shows the influence of ionic strength on the association of phenylalanyl-tRNA ligase and a 3/4 $tRNA^{Phe}$ obtained by splitting the $tRNA^{Phe}$ in the dihydrouridine loop. Its behaviour fits with the model, but the slope of the straight line indicates only 4 sites.

Although one must be very cautious in interpretating

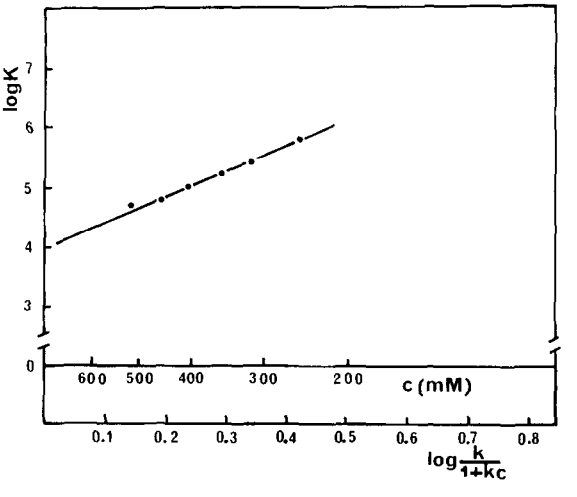

Fig.2. Effect of ionic strength on the association constant of a 3/4 $tRNA^{Phe}$ for phenylalanyl-tRNA ligase.

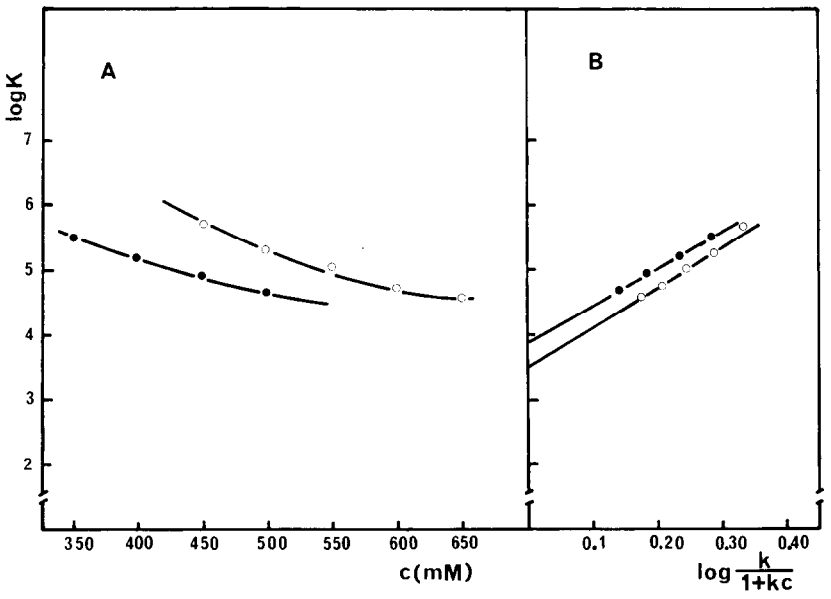

Fig.3. Effect of ionic strength on the association between $tRNA^{Phe}$ and phenylalanyl-tRNA ligase in the presence of methanol. The affinity constant of $tRNA^{Phe}$ for phenylalanyl-tRNA ligase in the absence (○) or in the presence of 20% methanol (●) as a function of ionic concentration (A) or $\log(k/1 + kc)$ (B).

such results, it is possible that 2 sites are located in the dihydrouracile arm or in the acceptor stem. These results also show that the affinity of the 3/4 $tRNA^{Phe}$ for phenylalanyl-tRNA ligase is still high.

It is known that organic solvents often increase the interactions between tRNA and aminoacyl-tRNA ligase ([1,2] and references therein). Aminoacylations were done in the presence of 20% methanol to determine whether this increase is due to an increase of the strength of the ionic interactions produced by the lowering of the dielectric constant of water [1] or an effect upon the structure of the tRNA [2].

Since $\log K$ varies with the reverse of the dielectric constant (ϵ) [4], the change in ϵ can be taken into account. Fig.3 shows that for a given ionic strength, methanol increases the affinity of $tRNA^{Phe}$ for phenylalanyl-tRNA ligase. The application of the polyelectrolyte law gives a straight line parallel to and slightly below that obtained in the presence of methanol. Thus the change in the behaviour of the system can be interpreted as a slight reduction of non ionic interactions. The slope of the new straight line still corresponds to 6 sites of ionic interactions. Thus the increase of the affinity of $tRNA^{Phe}$ for its cognate aminoacyl-tRNA ligase can be simply explained by an enhancement of ionic interactions.

Acknowledgements

The authors are grateful to Professor J. P. Ebel for his guidance in the preparation of this manuscript. We are also indebited to Professor Daune for his interest and advices concerning this work. We thank Mme M. Schlegel for her assistance in the isolation of the tRNAs and Mlle M. Delfau and Mme N. Ménard for their help in the preparation of the ligases. This work was partly supported by grants from the Délégation Générale à la Recherche Scientifique et Technique and from the Commissariat à l'Energie Atomique.

References

[1] Loftfield, R. B. (1972) Progr. Nucleic Acid Res. 12, 87–128.
[2] Yarus, M. (1972) Biochemistry 11, 2050–2060.

[3] Daune, M. (1969) Biopolymers 7, 659–670.
[4] Daune, M. (1972) Eur. J. Biochem. 26, 207–211.
[5] Poliakow, M. C., Champagne, M. C. and Daune, M. (1972) Eur. J. Biochem. 26, 212–219.
[6] Lagerkvist and Waldenström (1967) J. Biol. Chem. 242, 3021–3025.
[7] Fasiolo, F., Befort, N., Boulanger, Y. and Ebel, J. P. (1970) Biochim. Biophys. Acta 217, 305–318.
[8] Pearson, R. L., Weiss, J. F. and Kelmers, A. D. (1971) Biochim. Biophys. Acta 228, 770–774.
[9] Gilham, I., Millward, S., Blew, D., Von Tigerström, M., Wimmer, E. and Tener, G. M. (1967) Biochemistry 6, 3043–3056.
[10] Krebs, B., Werner, C. and Dirheimer, G. (1972) J. Eur. Tox. 6, 337–342.
[11] Yarus, M. and Berg, P. (1967) J. Mol. Biol. 28, 479–490.
[12] Yaniv, M. and Gros, F. (1969) J. Mol. Biol. 44, 17–30.
[13] Chousterman, S. and Chapeville, F. (1973) Eur. J. Biochem. 35, 51–56.
[14] Rouget, T. P. and Chapeville, F. (1971) Eur. J. Biochem. 4, 310–314.
[15] Gangloff, J. (1973) Thèse, Université Louis Pasteur, Strasbourg.
[16] Yarus, M. and Berg, P. (1970) Anal. Biochem. 35, 450–465.
[17] Fasiolo, F., Remy, P., Pouyet, J. and Ebel, J. P. (1974) Eur. J. Biochem. 50, 227–236.
[18] Goldstein, R. N., Stefanovic, S. and Kallenbach, N. R. (1972) J. Mol. Biol. 69, 217–236.
[19] Poletaev, A. I. (1973) Mol. Biol. 7, 84–92.
[20] Cole, P. E. and Crothers, D. M. (1972) Biochemistry 11, 4368–4375.
[21] Schreier, A. M. and Schimmel, P. R. (1974) J. Mol. Biol. 86, 601–620.
[22] Kim, S. H., Suddath, F. F., Quigley, G. J., Mc Pherson, A., Sussman, J. L., Wang, A. H. J., Seeman, N. C. and Rich, A. (1974) Science 185, 435–440.
[23] Klug, A., Robertus, J. D. Ladner, J.E., Brown, R. S. and Finch, J. T. (1974) Proc. Natl. Acad. Sci. USA 71, 3712–3715.
[24] Schuber, F. and Pink, M. (1974) Biochimie 56, 397–403.