COORDINATION OF Mn^{++} IONS AT CONTACT SITES BETWEEN tRNA AND AMINOACYL-tRNA SYNTHETASE

J.M. Backer, S.V. Vocel, L.M. Weiner, S.I. Oshevskii
and
O.I. Lavrik

Institute of Chemical Kinetics and Combustion of the Siberian Division of the Academy of Sciences of the USSR
and
Institute of Organic Chemistry of the Siberian Division of the Academy of Sciences of the USSR
Novosibirsk 90, USSR

Received February 24,1975

By means of PMR and ESR study the shielding of Mn^{++} ions by aminoacyl-tRNA synthetase has been detected in the aminoacyl-tRNA synthetase - tRNA complex at pH 7.5. At pH 6 this effect was not observed. We propose that ions interact with certain aminoacyl-tRNA synthetase groups protonated when passing to slightly acid pH. The role of Mn^{++} and Mg^{++} ions in the formation of a functionally active complex tRNA-aminoacyl-tRNA synthetase is discussed.

Information is needed on the nature of the contact sites between the enzyme and tRNA in a functionally active complex to understand the mechanism of tRNA recognition by aminoacyl-tRNA synthetase (amino acid: tRNA-ligase E.C. 6.11). With this aim in mind several attempts have been made to understand which parts of tRNA are protected by ARSase* from the nuclease hydrolysis and from complexing with complementary oligonucleotides (see reviews 1,2).

The present study is concerned with the protection of tRNA fragments with specific coordination sites of Mn^{++} by ARSase. It is known that tRNA has 5-7 strong sites of coordination of Mn^{++} and Mg^{++} [3] which are specifically distributed in the

* Abbreviations used: ARSase, aminoacyl-tRNA synthetase

tRNA macrostructure [4,5]. By means of NMR it was possible to observe sorption of low-molecular weight compounds on phosphate groupings near coordinated ions, as well as their interaction with an ion itself [6,7].

We studied the competition between ARSase and the low-molecular weight compounds at pH 6 and 7.5. The competition was observed only at pH 7.5. Similar results have been obtained by ESR study of the influence of ARSase on the interaction between Mn^{++} and NH_3^+-R radicals sorbed on phosphate groupings.

We propose that competition at pH 7.5 is caused by interaction of ions coordinated by tRNA with certain ARSase groups.

Experimental. E. Coli MRE-600 unfractionated tRNA was obtained according to ref.8. Crude tRNA contained 3% $tRNA^{Phe}$. The aminoacylation of tRNA was performed with ^{14}C-phenylalanine (220 Ci/mole, Chemapol.). Partially purified phenylalanine-tRNA synthetase of E. Coli MRE-600 was obtained according to ref.9. The enzyme preparation contained (in addition to phenylalanine-tRNA synthetase) some amounts of valyl-, lysile-, isolaucyl-, prolyl-tRNA synthetases. The enzyme and the enzyme-tRNA complexes were concentrated under vacuum in ultra-thimbles (Schleicher and Schüll) in a buffer tris HCl 0.05M, pH 7.5, or in sodium acetate 0.025M, pH 6. The concentration of the enzyme was equal to or greater than 10^{-4}M. The enzyme activity after concentration was controlled by measuring the rate of tRNA aminoacylation which was determined as described earlier [10]. The radioactivities were counted using a Mark II Nuclear Chicago Scintillation counter. The radical  was kindly given by T.Kukina (the Novosibirsk State University).

The PMR spectra were recorded using the JNM-4H-100 spectro-

meter. TMS was used as an external reference. All solutions were prepared in D_2O buffers. As the control experiments showed, the enzyme activity did not vary under conditions of the PMR experiment.

The ESR spectra were recorded using an E3-spectrometer at 77°K. In experiments at 77°K 40-50% glycerin were added to all solutions. According to the control experiments, in the presence of glycerin acceptor activity of the tRNA is constant while aminoacylation rate decreases by a factor of 3-4. To obtain the saturation curves at 77°K the ESR signal amplitude was determined between two certain points in the spectra of NH_3^+-R radical at various values for the amplitude of the radiofrequency field H_1. Location of these points which correspond to the points of the maximum inclination of the unsaturated signal was found with respect to the ESR signal of the powder sample of diphenylpycrylhydrazide. The linearity of the change of the radiofrequency field H_1 amplitude was controlled with respect to the unsaturating polycrystalline sample $CuSO_4$.

Results. It was found in refs 6,7 that Mn^{++} coordinated by tRNA caused a considerable broadening of the NMR signal of tetramethylammonium (TMA) and glycine. This broadening is due to the TMA binding to the phosphate group which is closest to the coordination ion[6] , and is also due to a simulataneous interaction of glycine with an ion and the nearest phosphate group[7].

The broadening observed is described for such systems by the following formula:

$$\Delta\nu = \frac{\Delta\nu_b \cdot K \cdot [Mn]_0}{1 + K \cdot [A]_0}$$

where: $\Delta\nu_b$ is the linewidth of the low-molecular weight compound caused by the coordinated ion Mn^{++} in the complex; $[A]_0$ is the concentration of the low-molecular weight compound; K is the stability constant of the complex under study, $[Mn]_0$ is

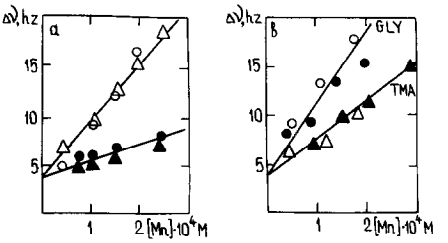

Fig. 1. The influence of aminoacyl-tRNA synthetase on the signal broadening of glycine and TMA at (a) pH=7.5 and (b) pH=6 in the tRNA-Mn^{++} (blank indications) and tRNA-Mn^{++}-ARSase system (shaded indications). The concentration of [tRNA] ≈ [ARSase] $=10^{-4}$M. ○, ● is glycine, △, ▲ is TMA.

---

the concentration of coordinated ions which cause the broadening [6,7].

It is noteworthy that the application of the two diamagnetic probes TMA and glycine allows us to observe easily the variation of the total parameter: the number of coordinated ions which cause the broadening when passing from tRNA to the tRNA-ARSase complex.

Experiments were performed under conditions of practically complete binding of Mn^{++} to tRNA and to the tRNA-ARSase complex. This was controlled by measuring the intensity of ESR signals of the non-complexed ions. It is seen from Fig.1a that at pH 7.5 the signal broadening of glycine and TMA is less by a factor of 3-3.5 in the tRNA-Mn^{++}-ARSase system than in the tRNA-Mn^{++} system. The similar influence of ARSase on the broadening of TMA and glycine implies that the number of coordinated Mn^{++} ions which cause signal broadening is decreased as a result of the formation of the tRNA-ARSase complex owing to sterical shielding by ARSase. However, such shielding is incomplete since in our experiments we did not observe any marked influence of ARSase on the signal broadening of water by coordinated ions.

As the experiments at pH 6 showed, ARSase practically did not affect the signal broadening of glycine, TMA (see Fig.1b), and water caused by coordinated ions. This result implies that at pH 6 there is no competition between ARSase and the low-molecular weight compounds for the binding sites near coordinated ions.

Similar results were obtained by an ESR study of the accessibility of Mn^{++} in the tRNA-ARSase complex. We studied the interaction of coordinated Mn^{++} with the iminoxyl radical NH_3^+-R by the method of stationary saturation of the radical ESR signal at 77°K in a water-glycerin matrice. According to ref.11, the radical saturation curves are shifted to the side of high amplitudes of the radiofrequency field H_1.

It is seen from Fig.2 that at pH 7.5 in the presence of tRNA containing coordinated Mn^{++}, the radical saturation curves are shifted to the side of high amplitudes of the radiofrequency field H_1.* However, tRNA complexes with Mn^{++} did not affect the saturation curves of the neutral radical O=⟨⟩N-Ȯ. This implies that the effects observed for NH_3^+-R are caused by an electrostatic sorption of radicals on the tRNA surface near coordinated Mn^{++}. In this case the influence of ARSase on the ra-

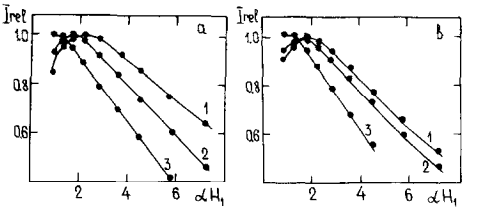

Fig. 2. The influence of ARSase on the shift of the saturation curves at (a) pH=7.5 and (b) pH=6. The concentration of [tRNA] ≈ [ARSase] ≈ 1.5×10^{-4}M. $[Mn^{++}]=5\times10^{-4}$M, $[R-NH_3^+]=5\times10^{-3}$M. Curve 1 - $R-NH_3^+$, curves 2 - $R-NH_3^+$ +tRNA-Mn^{++}-ARSase, curves 3- $R-NH_3^+$ +tRNA-Mn^{++}.

* In the absence of Mn^{++} tRNA did not affect the saturation curves.

dical saturation curves must be ascribed to the shielding of certain surface sites of tRNA with coordinated ions.

It was found that at pH 7.5 ARSase causes a partial shift of the saturation curve to the side with smaller values of H_1, while at pH 6 ARSase practically does not influence the saturation curves. This is an indication that at pH 7.5 coordinated ions are partially shielded by the enzyme.

Discussion. The competition between ARSase and the low-molecular weight compounds for the binding sites near Mn^{++} ions coordinated by tRNA is observed at pH 7.5. It implies that specific coordination sites of ions are shielded at the contact sites of tRNA with ARSase. These results are not striking since there is a definite correspondance between the location of specific coordination sites of Mn^{++} and Mg^{++} in tRNA [4,5] and the tRNA fragments which are in contact with ARSase (see reviews 1, 2), e.g., in the region of CCA-end and in the region near the thiouridylic residue.

Two mechanisms of shielding can be proposed: 1) owing to the presence of coordinated ions at long contact areas between tRNA and the enzyme, and 2) owing to the interaction of coordinated ions with certain ARSase groups. In the first case the absence of competition at pH 6 means that pH affects the location of coordinated sites of ions or the contact sites of tRNA with ARSase. However, at pH 7.5 and 6 in ile-$tRNA^{ile}$ from E.Coli there is a coordination site of Mg^{++} on the CCA-end which is in contact with aminoacyl-tRNA synthetase at pH 7.5, as well as at pH 6 (see ref.12). Similar results have been obtained for Mn^{++} in our preliminary experiments with phe-$tRNA^{phe}$ from E.Coli spin-labeled at α-amino group of aminoacyl residue.

If we assume that ion shielding in the tRNA complexed with

ARSase is due to the interaction of coordinated ions with certain groups of the enzyme, then the absence of the effects at pH 6 means the "switching off" of these groups from the interaction.

In general, imidazol, ionized sulphhydryl and carboxyl groups can interact with ions coordinated by tRNA. The idea of the interaction of carboxyl groups with Mn^{++} coordinated by tRNA has been recently suggested by Weiner et al.[7] for model systems: tRNA-Mn^{++}-amino acid (dipeptide). Obviously, the "switching off" of these groups from the interaction with ions coordinated by tRNA can proceed owing to protonating of weakly acid pH. The existence of groups with pK~6 was found by Helene et al.[13] for valyl-ARSase from E.Coli

The above considered ideas of the mechanism of ion shielding due to the interaction with certain "complementary" groupings of ARSase are more preferable as an explanation of our experimental data. In addition, a natural explanation can be given for the data on the role of ions in the process of tRNA recognition by ARSase if we assume that ions coordinated by tRNA interact with ARSase. In fact, within this assumption in a functionally active complex tRNA-ARSase, ions are needed only at certain pH to provide contacts between the certain fragments of tRNA and the enzyme.

As the analysis of literature data shows, in the transfer process of aminoacyl residue, i.e., in the process of recognition, Mg^{++} and Mn^{++} are needed at pH 7-8 [14,15] and refs 15-17, 19,20,22 in [15] , and are not needed at pH 5.5-7 [15,16] and refs 2,3,5-10,14 in [15] . If we assume that coordination ions react with the ionized groupings, it is easy to explain the possibility of substitution of diamines for ions in the transfer

process [14] by the ability of diamines to provide neutralization of anion groups and to involve them in the contact with certain tRNA fragments. In addition, incorrect aminoacylation detected in ref.17 at high ion concentrations can be a result of the formation of non-specific contacts between the anion groupings of ARSase and ions coordinated by phosphate groupings at the "weak" non-specific sites.

Acknowledgements. The authors are grateful to Prof. Yu.Molin for useful discussions and to S.Eremenko for his assistance in ESR experiments.

REFERENCES

1. Kisselev,L. & Favorova,O. (1974) in "Advances in Enzymology", v.4, 141-238.
2. Soll,D. & Schimmel,P. (1974) in "The Enzyme", v.10,489-537.
3. Danchin,A. (1972), Biopolymers 11, 1317-1333.
4. Vocel,S., Backer,J. & Slepneva,I. (1974) Biopolymers,in press.
5. Lynch,D. & Schimmel,P. (1974) Biochem., 13, 1841-1852.
6. Weiner,L., Backer,J. & Molin,Yu. (1973) FEBS Lett., 29,348-350.
7. Weiner,L., Backer,J. & Rezvukhin,A. (1974) Biochim.Biophys. Acta, in press.
8. Sandakchiev,L., Starostina,V., Stephanovich,L. & Chuchaev,V. (1967) Mol.Biol. (USSR), 1, 463-466.
9. Stulberg,M. (1967), J.Biol.Chem., 242, 1060-1064.
10. Kelmers,A., Novelli,G. & Stulberg,M. (1965) J.Biol.Chem., 240, 3979-3983.
11. Kulikov,A. & Likchtenshtein (1974), Biofizika (USSR), 19, 420-424.
12. Lynch,D. & Schimmel,P. (1974), Biochem., 13, 1852-1861.
13. Helene,C., Brun,F. & Yaniv,M. (1971) J.Mol.Biol.,58,349-365.
14. Chousterman,S. & Chapeville,F. (1973) Eur.J.Biochem., 35, 46-50.
15. Zimmerman,T. & Robison,B. (1972) Biochem.Biophys.Res.Commun., 47, 1138-1143.
16. Eldred,O. & Schimmel,P. (1972) Biochem., 11, 17-23.
17. Giege,R., Kern,D. & Ebel,J.P. (1972), Biochimie,54, 1245-1255.