CROATICA CHEMICA ACTA
CCACAA **77** (4) 599–604 (2004)
ISSN-0011-1643
CCA-2965
Original Scientific Paper

Stability of the Complex between Yeast Seryl-tRNA Synthetase and $tRNA^{Ser}$ under Different Electrophoretic Conditions

Ita Gruić-Sovulj,[a,b] Jasmina Rokov-Plavec,[a,b] Marko Močibob,[a,b] Tomislav Kamenski,[a] and Ivana Weygand-Đurašević[a,b,*]

[a]*Department of Chemistry, Faculty of Science, University of Zagreb, Strossmayerov trg 14, 10000 Zagreb, Croatia*

[b]*Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia*

RECEIVED JULY 27, 2003; REVISED NOVEMBER 13, 2003; ACCEPTED JANUARY 27, 2004

Key words
• aminoacyl-tRNA synthetase
• gel mobility shift assay
• zone-interference gel electrophoresis
• SerRS:$tRNA^{Ser}$ noncovalent complexes
• Mg^{2+} influence

Noncovalent interactions of yeast homodimeric seryl-tRNA synthetase (SerRS) and cognate $tRNA^{Ser}$ were studied by the gel mobility shift assay and zone-interference gel electrophoresis performed under the same binding and electrophoretic conditions. Purified $tRNA^{Ser}$ as well as total yeast tRNA were applied as ligands. In the absence of Mg^{2+}, SerRS:$(tRNA^{Ser})_1$ noncovalent complex was detected only by zone-interference gel electrophoresis. K_d values determined in the presence and absence of Mg^{2+} were in the same range, suggesting that Mg^{2+} ions mainly influence dissociation-association kinetics of the complex, with a minor contribution to its thermodynamic stability. Comparison of these two assays was shown to be useful in the analysis of thermodynamic and kinetic properties of protein:nucleic acid complexes.

INTRODUCTION

Noncovalent protein:nucleic acid interactions are often explored by gel electrophoresis under native conditions. Gel mobility shift assay[1,2] is based on the electrophoretic retardation of nucleic acid in the protein:nucleic acid complex relative to free nucleic acid and is usually performed under non-equilibrium conditions. In this assay, separation of the reaction components as they enter the gel and during electrophoresis perturbs the preexisting equilibrium, and favors the dissociation of the complex. Thus, the protein:nucleic acid complex dissociation in the gel (half-life time) is of greater importance than its thermodynamic stability (K_d). Complexes with equilibrium constants in the 10^{-6} mol dm^{-3} range can be resolved if their half-life time is on the same time-scale as electrophoresis.[3] On the other hand, short-living complexes that dissociate while entering the gel or during electrophoresis yield diffuse bands or cannot be detected.[4] Abrahams *et al.*[5] developed the zone-interference gel electrophoresis as a new method for studying short-lived protein:nucleic acid complexes under native equilibrium conditions. The principle of the method is that during electrophoresis a protein:nucleic acid complex is migrating all the time through a zone of known concentration of the nucleic acid ligand. In this way, dissociation is permanently counteracted by association under rapid dynamic equilibrium conditions. The method was developed to study noncovalent interactions between the EF-Tu:guanine-nucleotide:aurodox complex and tRNA[5] and it was used for further investigation of EF-Tu:tRNA interactions.[6–8]

* Author to whom correspondence should be addressed. (E-mail: weygand@rudjer.irb.hr)

Aminoacyl-tRNA synthetases (aaRS) are a group of enzymes involved in protein biosynthesis that catalyze aminoacylation of a tRNA by its cognate amino acid in a two-step reaction. The enzyme first activates the amino acid using Mg^{2+}·ATP to form the enzyme-bound aminoacyl-adenylate intermediate. In the second step, the amino acid is transferred to the cognate tRNA. Basically, each aaRS is specific for one amino acid and a set of cognate tRNAs, known as isoacceptors. Seryl-tRNA synthetases (SerRS) from *Thermus thermophilus* and *Escherichia coli* form two types of noncovalent complexes with cognate $tRNA^{Ser}$; SerRS:$(tRNA^{Ser})_1$ and SerRS:$(tRNA^{Ser})_2$.[9–12] Both types of noncovalent complexes from *E. coli* were detected by zone-interference gel electrophoresis[9] under the conditions described by Abrahams *et al*.[5–7] The tRNA is bound across the two subunits of dimeric SerRS, as revealed by the SerRS:$(tRNA^{Ser})_1$ crystal structure[10] and biochemical studies on heterodimers.[13] SerRS from the yeast *Saccharomyces cerevisiae* has also been found[14–17] to bind one or two $tRNA^{Ser}$. SerRS:$(tRNA^{Ser})_2$ complex observed by MALDI-MS[16] could not be detected by the gel mobility shift assay despite variations in the buffer composition, pH and ionic strength. This complex was electrophoretically detected only after covalent cross-linking[17] of SerRS and its cognate $tRNA^{Ser}$.

Metal ions are well known cofactors in many biochemical reactions and processes. The Mg^{2+} ions are involved in the reaction of the aminoacyl-tRNA synthetases through binding to ATP, tRNA or the enzyme itself. Several Mg^{2+} are known to be bound to the tRNA but only a few are functionally of major importance for maintaining a correct conformation of tRNA.[18] In phenylalanyl-tRNA synthetase, two magnesium-binding sites have been shown in the enzyme.[19] The crystal structure of the SerRS:ATP complex[20] from *Thermus thermophilus* reveals three Mn^{2+}. One of them binds to the α-phosphate and β-phosphate of ATP, and the other two to the β-phosphate and γ-phosphate. The same is believed to be true of magnesium ions as well.

In this paper, we present a somewhat modified zone-interference gel electrophoretic method in terms of the electrophoretic conditions and the gel system used. The basic idea was to find a way of direct comparison of aaRS:tRNA complex stability and stoichiometry under the equilibrium and non-equilibrium electrophoretic conditions. Therefore, gel mobility shift analysis and zone-interference gel electrophoresis were both performed on polyacrylamide gels under the same binding and electrophoretic conditions (buffer composition and pH, ionic strength, potential difference). Zone-interference gel electrophoresis allows easy determination of the K_d value for the cognate aaRS:tRNA complex, but requires a huge amount of purified nucleic acid as a ligand.[9] Here we describe a successful use of total tRNA as a ligand. Moreover, by this assay we determined K_d's of the SerRS:$(tRNA^{Ser})_1$ complex in the presence and in the absence of Mg^{2+} ions, and indicated that Mg^{2+} ions mainly influenced the dissociation-association of the complex.

EXPERIMENTAL

General

Total yeast tRNA was purchased from Roche Molecular Biochemicals.

Purification of Native $tRNA^{Ser}$

To isolate $tRNA^{Ser}$ isoacceptors, total brewer's yeast tRNA was serylated using pure SerRS, derivatized by naphtoxyacetylation and purified by chromatography on a benzoylated DEAE-cellulose column, as described previously.[16] Purified $tRNA^{Ser}$ accepted at least 1.2 nmol of serine/A_{260} unit of tRNA in the standard aminoacylation assay.[21] Pure $tRNA^{Ser}$ was subjected to MALDI-MS analysis.[22] The resolution of $m/\Delta m$ = 170, achieved in the linear mode of operation, confirmed the high purity level of the sample.

Renaturation of tRNA

All tRNAs used in noncovalent interaction studies were carefully renatured immediately before complex formation (gel mobility shift assay) or loading on the gel (zone-interference gel electrophoresis). 50 μl aliquots were incubated in the water-bath at 80 °C and the temperature was allowed to decrease to 50 °C. Then $MgCl_2$ was added to the final concentration of 10 mmol dm^{-3}, and the tRNA sample was cooled slowly to 30 °C. When tRNA samples were prepared for the electrophoresis in the absence of Mg^{2+}, the final concentration of $MgCl_2$ in renaturation mixture was 1 mmol dm^{-3} to ensure a proper folding of tRNA.

Overexpression and Purification of SerRS

Gene (*SES1*) for yeast cytosolic SerRS was cloned into pCJ11. Yeast strain overproducing SerRS was generated by transformation of *S. cerevisiae* S2088 with plasmid pCJ11*SES1*. The transformants were grown in a glucose selective medium at 30 °C until saturation, and were then diluted 25-fold with a galactose-containing medium for promoter induction. SerRS was purified by the two-step chromatographic procedure on FPLC MonoQ and MonoS columns as described previously.[23]

Gel Mobility Shift Assay

Gel mobility shift assay in the presence of Mg^{2+} ions was performed as described previously.[17]

When the gel mobility shift assay was performed in the absence of Mg^{2+} ions, the Mg^{2+}concentration was lowered and EDTA was added. Purified native $tRNA^{Ser}$, renatured in the presence of 1 mmol dm^{-3} $MgCl_2$, was incubated with the enzyme for 5 min at 30 °C in a binding buffer containing 30 mmol dm^{-3} imidazol pH 8.0 and 1 mmol dm^{-3}

Na_2EDTA, followed by cooling on ice. The resulting concentration of Mg^{2+} in the reaction mixture was 0.2 mmol dm^{-3}. Glycerol was added to the final concentration of 7.5 % and the preformed complex was subjected to electrophoresis on 6 % acrylamide/bisacrylamide (40:1) gel in electrophoresis buffer (50 mmol dm^{-3} Tris, 25 mmol dm^{-3} boric acid, 1 mmol dm^{-3} Na_2EDTA; pH 8.0). Electrophoresis was carried out at 4 °C for 3–4 h at 14 V cm^{-1}. EDTA was added to sequester the divalent cation traces present in binding and electrophoresis buffers. Gels were stained by the standard silver staining procedure.

Zone-interference Gel Electrophoresis

A series of zone solutions (210 µl) of increasing tRNA concentration in the 30 mmol dm^{-3} imidazol pH 8.0, 10 mmol dm^{-3} $MgCl_2$, 4 % glycerol were applied discontinuously on the gel. First, 30 µl of tRNA zone was brought into the well and 5 µl of SerRS in 30 mmol dm^{-3} imidazol pH 8.0, 10 mmol dm^{-3} $MgCl_2$, 12.5 % glycerol was layered underneath the tRNA zone solution. Then, 6×30 µl of tRNA zones were applied every 15 minutes. Discontinuous application of the zones did not result in a discontinuous movement of the tRNA band in the gel; a continuous tRNA zone was formed, as revealed by toluidine staining (data not shown). tRNA concentration in zones was varied from 0–204 µmol dm^{-3} when the total yeast tRNA was used as a ligand (corresponding to 0–12 µmol dm^{-3} $tRNA^{Ser}$ isoacceptors). Electrophoresis was performed on 6 % acrylamide/bisacrylamide gels (40:1) at 14 V cm^{-1}, 4 °C, for approximately 2 hours. Electrophoretic buffer was 50 mmol dm^{-3} Tris, 25 mmol dm^{-3} boric acid, 10 mmol dm^{-3} magnesium acetate; pH 8.0. Gels were stained with Coomassie brilliant blue. When zone-interference gel electrophoresis was performed in the absence of Mg^{2+} ions, magnesium was omitted from all solutions used and EDTA was added to the final concentration of 1 mmol dm^{-3}.

RESULTS AND DISCUSSION

In order to investigate the direct dependence of aaRS:tRNA complex stability upon the nucleic acid ligand availability, zone-interference gel electrophoresis has been performed under the same binding and electrophoresis conditions as used in the gel mobility shift assay. According to the original method,[5] protein is migrating continuously through the 210 µl zones of increasing tRNA concentrations on 1.5 % agarose gel. In our hands, electrophoresis performed on polyacrylamide gels resulted in improved resolution compared to the one performed on agarose gels. Migration of the 210 µl tRNA zones on polyacrylamide gel was checked by toluidine staining. All tRNA zones appeared as broad bands of a 4–5 cm length (data not shown). tRNA was renatured in the same way as for the mobility shift assay and then the same buffer, as the one used for complex formation in the mobility shift, was added to the aliquots of SerRS and renatured tRNA. Electrophoresis buffer was the same in both assays.

SerRS:$(tRNA^{Ser})_2$ Complex could not Be Detected by Zone-interference Gel Electrophoresis

We have recently reported[17] that yeast SerRS binds two $tRNA^{Ser}$ molecules in an anticooperative way. While SerRS:$(tRNA^{Ser})_1$ complex is easily observed by the gel mobility shift assay (Figure 1A), the complex of dimeric enzyme with two $tRNA^{Ser}$ molecules cannot be detected despite variations in buffer pH and ionic strength; it is detected only after stabilization by covalent cross-linking. In order to detect the SerRS:$(tRNA^{Ser})_2$ complex on the native gel without covalent stabilization, yeast SerRS:$tRNA^{Ser}$ interactions were explored by zone-interference gel electrophoresis. Figure 2A shows the electrophoresis on 6 % polyacrylamide gel with yeast SerRS migrating through the zones of increasing total yeast tRNA concentrations (0–204 µmol dm^{-3}). Concentration of $tRNA^{Ser}$ isoacceptors in the applied total tRNA zones was 0–12 µmol dm^{-3}. Not even under the equilibrium conditions in the zone-interference gel electrophoresis did we succeed in detecting two types of complexes between SerRS and $tRNA^{Ser}$. Diffuse bands appearing in lanes 2, 3 and 4 are probably a consequence of the non-rapid dissociation kinetics of SerRS:$(tRNA^{Ser})_1$ complex, since it is known that rapid complex dissociation/association is a prerequisite for obtaining sharp bands in the equilibrium native electrophoresis. In our hands, the described band pattern was present only in the zones of a low tRNA concentration. Determination of the K_d value for the SerRS:$(tRNA^{Ser})_1$ com-

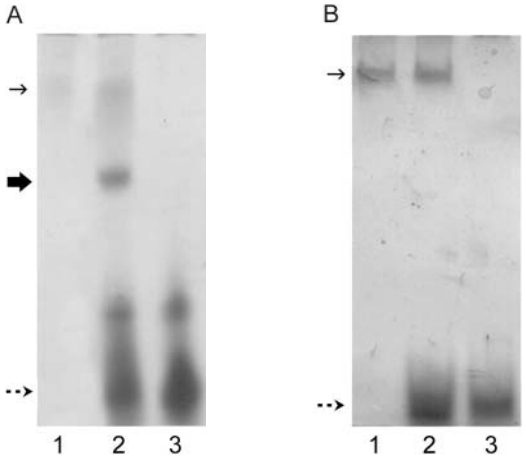

Figure 1. Yeast SerRS:tRNA interactions explored by the gel mobility shift assay. SerRS and tRNA were detected by the standard silver staining procedure. Full line arrow, SerRS; bold arrow, SerRS:$tRNA^{Ser}$ complex; dashed line arrow, uncomplexed tRNA. (A) Gel mobility shift assay performed in the presence of Mg^{2+}. Lanes: 1) 10 pmol of SerRS, 2) 20 pmol of $tRNA^{Ser}$ and 10 pmol of SerRS, 3) 20 pmol of $tRNA^{Ser}$. The band migrating between noncomplexed tRNA and the SerRS:tRNA complex can be assigned to tRNA oligomers.[17] (B) Gel mobility shift assay performed in the absence of Mg^{2+}. Lanes: 1) 10 pmol of SerRS, 2) 20 pmol of $tRNA^{Ser}$ and 10 pmol of SerRS, 3) 20 pmol of $tRNA^{Ser}$. Binding and electrophoretic buffers were deprived of magnesium ions and contained 1 mmol dm^{-3} Na_2EDTA.

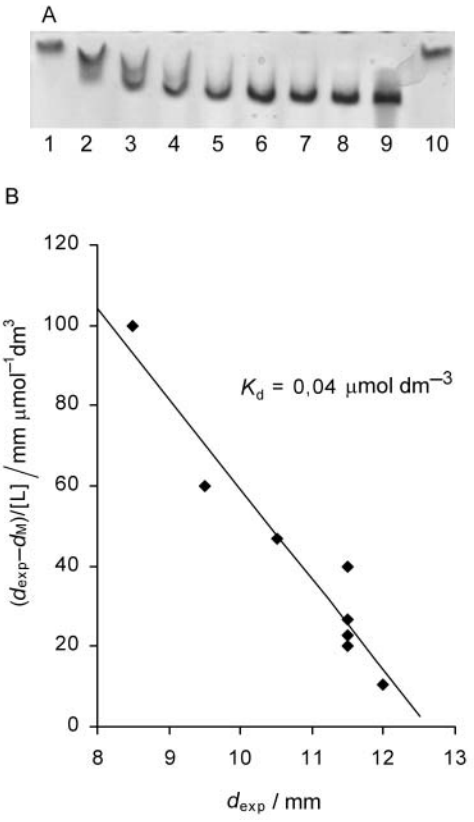

Figure 2. Yeast SerRS:tRNA interactions explored by the zone-interference gel electrophoresis. (A) Each lane contained 5 μl yeast SerRS (5 μM). Lane 1 represents the SerRS movement in the absence of tRNA. Lanes 2–9 represent the SerRS movement in 210 μl of the total yeast tRNA ligand zones of different concentration. Concentration of $tRNA^{Ser}$ isoacceptors in the applied total tRNA zones were 0.1, 0.2, 0.4, 0.8, 1.5, 3, 6 and 12 μM (lanes 2–9, respectively). Lane 10 represents the SerRS movement in 210 μl native yeast $tRNA^{Tyr}$ zone (5 μM). SerRS was detected by Coomasie brilliant blue staining. (B) Determination of K_d values for the yeast SerRS:$(tRNA^{Ser})_1$ complex using experimental data shown in (A), according to the equation[5] $(d_{exp}\text{-}d_M)/[L] = -(1/K_d)d_{exp} + d_{ML}/K_d$. d_{exp} is the migration distance of SerRS in the tRNA zone; d_M is the migration distance of the free SerRS; [L] is concentration of $tRNA^{Ser}$ isoacceptors in the zone; d_{ML} is the migration distance of the SerRS:$tRNA^{Ser}$ complex.

plex was not very precise due to band diffusion (Figure 2A). Estimated K_d value is in the 10^{-7} mol dm^{-3} range (Figure 2B), which is comparable to the previously published results obtained by the analytical sedimentation technique.[14] Since it is known that less stable noncovalent complexes are more easily detected by electrophoresis performed under equilibrium than non-equilibrium conditions,[5] inability to detect SerRS:$(tRNA^{Ser})_2$, even by the zone-interference gel electrophoresis, confirms that SerRS binds the second $tRNA^{Ser}$ with a significantly lower affinity than the first $tRNA^{Ser}$. On the other hand, this result together with our previous findings[17] shows that some protein:nucleic acid interactions could escape detection by equilibrium or non-equilibrium electrophoretic methods and could be demonstrated only by covalent stabilization.

K_d value for the SerRS:$(tRNA^{Ser})_1$ complex obtained in the presence of total tRNA as a ligand (10^{-7}–10^{-8} mol dm^{-3}, Figure 2B) is in good agreement with previously published data[14] ($1/K_d > 10^6$ mol^{-1} dm^3). This suggests that specific noncovalent interactions between synthetase and cognate tRNA could be investigated qualitatively and quantitatively by using total tRNA as a ligand. No change in SerRS mobility was detected in the zone of non-cognate $tRNA^{Tyr}$ (Figure 2A, lane 10), showing that the effects observed were not caused by nonspecific interactions with non-cognate tRNAs present in total RNA. However, a small contribution of non-specific interactions between SerRS and non-cognate tRNA present in total tRNA could not be absolutely excluded from the overall effect. Since it is known that a large amount of purified tRNA is necessary for determination of K_d for the aaRS:tRNA complex by zone-interference gel electrophoresis,[9] the ability to measure K_d values using total cellular tRNA is a valuable contribution to the original method.

Mg^{2+} Ions Influence Dissociation-Association Kinetics of the SerRS:$(tRNA^{Ser})_1$ Complex

In the absence of Mg^{2+} ions, the SerRS:$(tRNA^{Ser})_1$ noncovalent complex was not detected by the gel mobility shift assay (Figure 1B). There are two possibilities: either the complex is not formed in the absence of Mg^{2+} or the formed complex is not stable enough for non-equilibrium electrophoretic detection. To investigate whether Mg^{2+} ions influence complex formation or complex stability, we have performed an equilibrium assay. tRNA renaturation and complex formation procedures, as well as the running buffer were the same as in the mobility shift performed in the absence of Mg^{2+}. Figure 3A shows that the noncovalent complex between the yeast SerRS and $tRNA^{Ser}$ is formed in the absence of Mg^{2+} ions, and can be detected by zone-interference gel electrophoresis. Total yeast tRNA was used as a ligand and the concentration of $tRNA^{Ser}$ isoacceptors was 0–12 μmol dm^{-3}. The K_d value determined for the SerRS:$(tRNA^{Ser})_1$ complex in the absence of Mg^{2+} was in the same range (Figure 3B) as K_d determined in the presence of Mg^{2+} ions (Figure 2B), suggesting that Mg^{2+} ions mainly influence dissociation-association kinetics of the SerRS:$(tRNA^{Ser})_1$ complex. This also indicates that in the presence of Mg^{2+} the SerRS:$(tRNA^{Ser})_1$ noncovalent complex has a longer dissociation half-life time, allowing its detection by the gel mobility shift assay.

It is well evidenced in the literature that Mg^{2+} ions are of great importance in stabilizing the tRNA tertiary structure.[24,25] It is also known that Mg^{2+} could influence the aaRS:tRNA complex formation.[14,15,26,27] Fluorescence quenching of the yeast SerRS[15] by $tRNA^{Ser}$ was negligible in the absence of Mg^{2+}, and the same was demonstrated[26] for human aspartyl-tRNA synthetase and non-

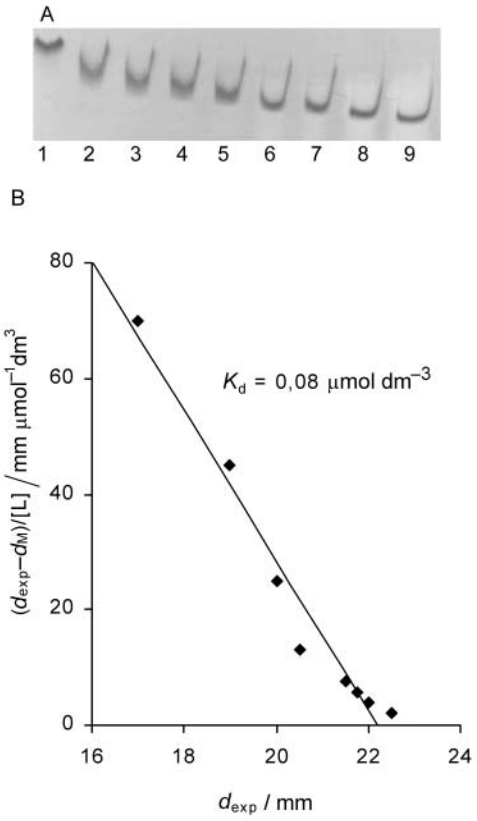

Figure 3. Yeast SerRS:tRNA interactions explored by the zone-interference gel electrophoresis in the absence of Mg^{2+}. (A) Each lane contained 5 μl yeast SerRS (5μM). Lane 1 represents the SerRS movement in the absence of tRNA. Lanes 2–9 represent the SerRS movement in 210 μl of the total yeast tRNA ligand zones of different concentration. Concentration of $tRNA^{Ser}$ isoacceptors in the applied total tRNA zones were 0.1, 0.2, 0.4, 0.8, 1.5, 3, 6 and 12 μM (lanes 2–9, respectively). Binding and electrophoretic buffers were deprived of magnesium acetate and contained 1 mmol dm^{-3} Na_2EDTA. SerRS was detected by Coomasie brilliant blue staining. (B) Determination of K_d values for the yeast SerRS:$tRNA^{Ser}$ complex using experimental data shown in (A). For explanation of symbols used see legend to Figure 2B.

cognate $tRNA^{fMet}$. It is tempting to speculate that the presence of Mg^{2+} ions mainly influences association-dissociation kinetics of the SerRS:$(tRNA^{Ser})_1$ complex with a minor effect on its thermodynamic stability (K_d). One explanation could possibly be that in the absence of Mg^{2+} tRNA does not fit precisely in the tRNA binding site, due to a somewhat altered SerRS:$tRNA^{Ser}$ interaction.[28] Imprecise binding of tRNA could influence rapid dissociation of the noncovalent complex formed. On the other hand, it is known that the main contribution to the total tRNA:SerRS binding energy[10] is the interaction between the α-helical coiled-coil of the SerRS and the variable arm of $tRNA^{Ser}$, the main identity element of $tRNA^{Ser}$. It seems that in the noncovalent SerRS:$(tRNA^{Ser})_1$ complex, detected under the equilibrium conditions in the absence of Mg^{2+}, the coiled-coil:variable arm interaction is preserved, leading to similar K_d values in the absence and presence of Mg^{2+}.

These results show that Mg^{2+} ions are important in maintaining the kinetically stable SerRS:$(tRNA^{Ser})_1$ noncovalent complex. Whether the kinetically less stable SerRS:$(tRNA^{Ser})_1$ complex obtained in the absence of Mg^{2+} is an efficient macromolecular complex in aminoacylation reaction should be explored by a different experimental approach. Further, detection of SerRS:$tRNA^{Ser}$ noncovalent interactions in the absence of Mg^{2+} ions only by the zone-interference gel electrophoresis supports the usage of equilibrium electrophoretic conditions when apparently no interaction is observed under the non-equilibrium ones.

Abbreviations. – aaRS – aminoacyl-tRNA synthetase; SerRS – seryl-tRNA synthetase; aaRS:tRNA – noncovalent complex between synthetase and tRNA; MALDI-MS – Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

Acknowledgements. – We thank Irena Landeka for her assistance with the tRNA *in vitro* transcriptions. This work was supported by grants from the International Centre for Genetic Engineering and Biotechnology, Trieste, the Ministry of Science and Technology of the Republic of Croatia, and the National Institutes of Health (NIH/Firca).

REFERENCES

1. M. G. Fried and D. M. Crothers, *Nucleic Acids Res.* **9** (1981) 6505–6525.
2. M. M. Garner and A. Revzin, *Nucleic Acids Res.* **9** (1981) 3047–3060.
3. A. Revzin, J. Ceglarek, and M. Garner, *Anal. Biochem.* **153** (1986) 172–177.
4. M. G. Fried, *Electrophoresis* **10** (1989) 366–376.
5. J. P. Abrahams, B. Kraal, and L. Bosch, *Nucleic Acids Res.* **16** (1988) 10099–10108.
6. J. P. Abrahams, B. Kraal, B. F. Clark, and L. Bosch, *Nucleic Acids Res.* **19** (1991) 553–557.
7. J. P. Abrahams, M. J. van Raaij, G. Ott, B. Kraal, and L. Bosch, *Biochemistry* **30** (1991) 6705–6710.
8. L. Krasny, J. R. Mesters, L. N. Tieleman, B. V. Fucik, R. Hilgenfeld, and J. Jonak, *J. Mol. Biol.* **283** (1998) 371–381.
9. F. Borel, C. Vincent, R. Leberman, and M. Härtlein, *Nucleic Acids Res.* **22** (1994) 2963–2969.
10. V. Biou, A. Yaremchuk, M. Tukalo, and S. Cusack, *Science* **263** (1994) 1404–1410.
11. A. D. Yaremchuk, M. A. Tukalo, I. Krikliviy, N. Malchenko, V. Biou, C. Berthet-Colominas, and S. Cusack, *FEBS Lett.* **310** (1992) 157–161.
12. S. Price, S. Cusack, F. Borel, C. Berthet-Colominas, and R. Leberman, *FEBS Lett.* **324** (1993) 167–170.
13. C. Vincent, F. Borel, J. C. Willison, R. Leberman, and M. Härtlein, *Nucleic Acids Res.* **23** (1995) 1113–1118.
14. G. Krauss, A. Pingoud, D. Boehme, D. Riesner, F. Peters, and G. Maass, *Eur. J. Biochem.* **55** (1975) 517–529.
15. R. Rigler, U. Pachmann, R. Hirsch, and H. G. Zachau, *Eur. J. Biochem.* **65** (1976) 307–315.
16. I. Gruić-Sovulj, H. C. Lüdemann, F. Hillenkamp, I. Weygand-Đurašević, Ž. Kućan, and J. Peter-Katalinić, *J. Biol. Chem.* **272** (1997) 32084–32091.

17. I. Gruić-Sovulj, I. Landeka, D. Söll, and I. Weygand-Đurašević, *Eur. J. Biochem.* **269** (2002) 5271–5279.
18. V. Serebrov, R. J. Clarke, H. J. Gross, and L. Kisselev, *Biochemistry* **40** (2001) 6688–6698.
19. J. Pimmer and E. Holler, *Biochemistry* **18** (1979) 3714–3723.
20. H. Belrhali, A. Yaremchuk, M. Tukalo, C. Berthet-Colominas, B. Rasmussen, P. Bösecke, O. Diat, and S. Cusack, *Structure* **3** (1995) 341–352.
21. B. Lenhard, S. Filipić, I. Landeka, I. Škrtić, D. Söll, and I. Weygand-Đurašević, *J. Biol. Chem.* **272** (1997) 1136–1141.
22. I. Gruć-Sovulj, H.-C. Lüdemann, F. Hillenkamp, I. Weygand-Đurašević, Ž. Kućan, and J. Peter-Katalinić, *Nucleic Acids Res.* **25** (1997) 1859–1861.
23. I. Weygand-Đurašević, N. Ban, D. Jahn, and D. Söll, *Eur. J. Biochem*. **214** (1993) 869–877.
24. E. J. Maglott and G. D. Glick, *Nucleic Acids Res.* **25** (1997) 3297–3301.
25. K. N. Nobles, C. S. Yarian, G. Liu, R. H. Guenther, and P. F. Agris, *Nucleic Acids Res.* **30** (2002) 4571–4760.
26. R. Hammamieh and D. C. H. Yang, *J. Biol. Chem.* **276** (2001) 428–433.
27. U. Pachmann, E. Cronvall, R. Rigler, R. Hirsch, W. Wintermeyer, and H. G. Zachau, *Eur. J. Biochem.* **39** (1973) 265–273.
28. U. Pachmann and H. G. Zachau, *Nucleic Acids Res.* **5** (1978) 961–973.

SAŽETAK

Stabilnost kompleksa kvaščeve seril-tRNA-sintetaze i $tRNA^{Ser}$ pod različitim elektroforetskim uvjetima

Ita Gruić-Sovulj, Jasmina Rokov-Plavec, Marko Močibob, Tomislav Kamenski i Ivana Weygand-Đurašević

Nekovalentne interakcije između homodimerne seril-tRNA sintetaze (SerRS) iz kvasca i pripadne $tRNA^{Ser}$ istraživane su gel retardacijskom i zonskom interferencijskom elektroforezom, provedenim u istim eksperimentalnim uvjetima. Kao ligandi rabljeni su pročišćena $tRNA^{Ser}$ i ukupna tRNA iz kvasca. U odsutnosti Mg^{2+} nekovalentni kompleks SerRS:$(tRNA^{Ser})_1$ opažen je samo zonskom interferencijskom elektroforezom. Sličnost konstanti disocijacija određenih u prisutnosti i odsutnosti Mg^{2+} upućuje da magnezijevi ioni pretežno utječu na kinetiku asocijacije i disocijacije kompleksa, s neznatnim utjecajem na njegovu termodinamičku stabilnost. Usporedba rezultata dviju metoda pokazala se upotrebljivom u analizi termodinamičkih i kinetičkih svojstava kompleksa između proteina i nukleinskih kiselina.