INTERACTIONS OF SERYL-tRNA SYNTHETASE WITH SERINE AND PHENYLALANINE SPECIFIC tRNA

R.RIGLER, E.CRONVALL
Institute for Cell Research, Medicinska Nobel Institutet, Karolinska Institutet, Sweden

and

R.HIRSCH, U.PACHMANN and H.G.ZACHAU
Institut für Physiologische Chemie und Physikalische Biochemie der Universität München, Germany

Received 5 November 1970

1. Introduction

The interactions of tRNAs with their cognate aminoacyl tRNA synthetases are specificity determining steps in protein biosynthesis. These interactions have been investigated by various methods (summaries [1–3]). Fluorescence methods as applied in titration experiments [4, 5] have the advantage of great sensitivity and interfere least with the state of equilibrium. Newly developed fluorescence temperature jump techniques [6] allow also the kinetics of fast reactions between the two macromolecules to be followed.

We report on fluorescence titration, temperature jump, and stopped flow experiments with seryl-tRNA synthetase (SRS) and various tRNAs. The results contribute to the understanding of the specificity of the recognition process.

2. Material and methods

2.1. *Synthetase and tRNA*

SRS activity [7] was assayed by incubating for 30 min at 37° in 0.1 ml: 20 A_{260} units unfractionated yeast tRNA, 2.5 nmoles ^{14}C-Ser, 0.5 μmole ATP, 5 μmoles tris-HCl pH 7.5, 1.5 μmoles $MgCl_2$, 2 μg serum albumin and varying amounts of the synthetase preparations. After trichloroacetic acid precipitation the radioactivity was determined on filter discs. 1 unit of synthetase incorporates 1 μmole Ser/min under these conditions.

SRS was prepared from brewer's yeast by a procedure [7] modified from [8]. The enzyme was 2000-fold enriched and had 108 mU./mg protein (protein determined by the method of Lowry et al. [9] with serum albumin as standard). Contamination by Phe-tRNA synthetase was less than 3%. According to disc electrophoresis under dissociating and non-dissociating conditions SRS is a dimeric enzyme with a molecular weight of about 100,000 [7]. The purity of SRS according to disc electrophoresis was at least 90%. In all physical measurements the synthetase activity remained until the end above 95%.

$tRNA^{Ser}$ and $tRNA^{Phe}$ were purified from brewer's yeast tRNA of Boehringer Mannheim GmbH by standard procedures [10, 11]. There was no mutual contamination between the two tRNAs according to oligonucleotide analyses. Yeast $tRNA^{Val}$ was donated by U.Lagerkvist in a collaborative effort.

2.2. *Fluorescence titrations*

SRS was titrated in 0.03 M K phosphate, pH 7.3, 0.5 mM EDTA, 1 mM GSH, 10% (V) glycerol. The fluorescence was excited at 280 nm and the emission, after passing a WG 320 and UG 11 filter (Schott and Gen.), was observed at right angles. The intensity of the exciting radiation was recorded simultaneously with the fluorescence emission using a beam splitting arrangement to correct for intensity fluctuations. A X–Y recorded with a time basis was used to control the final state of equilibrium after each titration step as well as the time of ilumination, since prolonged

 North-Holland Publishing Company – Amsterdam

and intense irradiation was found to inactivate the enzyme.

2.3. *Correction for tRNA absorption*

The enzyme fluorescence was corrected for the presence of an additional absorbing and non-emitting species according to:

$$F_{\text{corrected}} = F_{\text{measured}} \cdot \frac{k_1+k_2}{k_1} \cdot \frac{1-e^{-k_1 a}}{1-e^{-(k_1+k_2)a}}$$

where $k_1 a$ and $k_2 a$ = 2.3 A_{280} of enzyme and tRNA, respectively, measured at the pathlength a. Since it can be shown that the solid angle of observation affects the correction term [12] the aperture of the emitting radiation was kept to a minimum.

2.4. *Fluorescence relaxation and stopped flow measurements*

SRS was used in the above buffer and in 0.02 M ammonium cacodylate pH 7.2, 0.1 M $(NH_4)_2SO_4$, 5 mM EDTA, 15 mM $MgCl_2$ and 1 mM GSH. The instruments used were designed to record intensity and polarisation of fluorescence after a temperature pulse [6] and stopped flow mixing of volumes down to 50 µl [13]. The signal was measured as the difference between fluorescence signal and reference signal from the exciting light beam to minimize lamp fluctuation. For each concentration investigated new enzyme and tRNA were used.

3. Results

The fluorescence of SRS originates mainly from tryptophan residues since the emission maximum was found at 340 nm. This fluorescence was quenched when $tRNA^{Ser}$ or $tRNA^{Phe}$ was added (fig. 1). Addition of $tRNA^{Val}$ had an effect on SRS fluorescence only at the lowest temperature; at the two higher temperatures the fluorescence remained completely unchanged (experiment not shown). Preliminary experiments with phenylalanyl-tRNA synthetase showed that its fluorescence is also influenced by all 3 tRNAs; on addition of $tRNA^{Ser}$ and $tRNA^{Val}$ fluorescence increases and subsequent decreases were observed under certain conditions. Probably all titration curves result from a superposition of fluorescence quenching

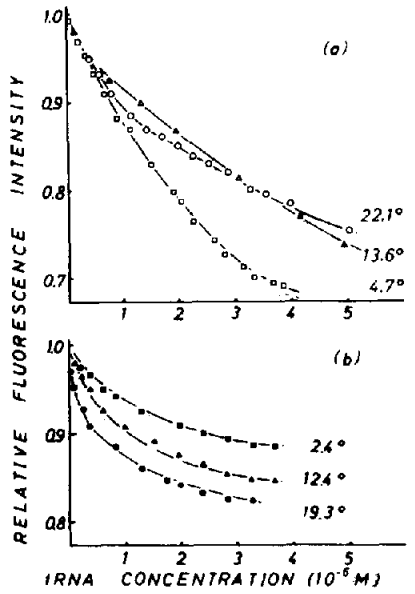

Fig. 1. Fluorescence titration of SRS with $tRNA^{Ser}$ (a) and $tRNA^{Phe}$ (b). The SRS concentration was kept at 1.5×10^{-6} M during the whole experiment.

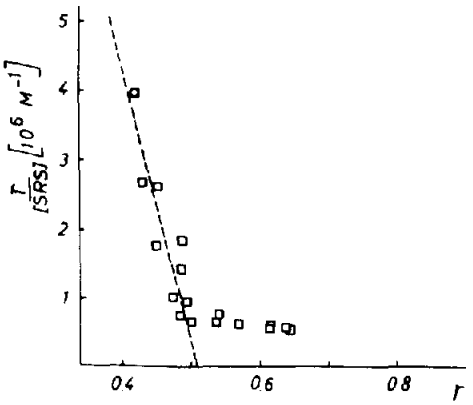

Fig. 2. Scatchard plot of the data from fig. 1a for the binding of $tRNA^{Ser}$ to SRS at 4.7°. r is the number of moles of SRS bound per mole of tRNA. (SRS) is the concentration of free enzyme.

and enhancing processes. Work on curve fitting computer programs is in progress.

Analysis of a SRS $tRNA^{Ser}$ quenching curve according to Scatchard indicated the occurrence of at least two binding processes (fig. 2), one of which extrapolates to a saturation number (n) of 2 moles tRNA per mole enzyme with an intrinsic association constant (K_{ass}) of 5×10^7 M^{-1}. A second process with a much lower affinity points to n = 1. Also in the SRS $tRNA^{Phe}$

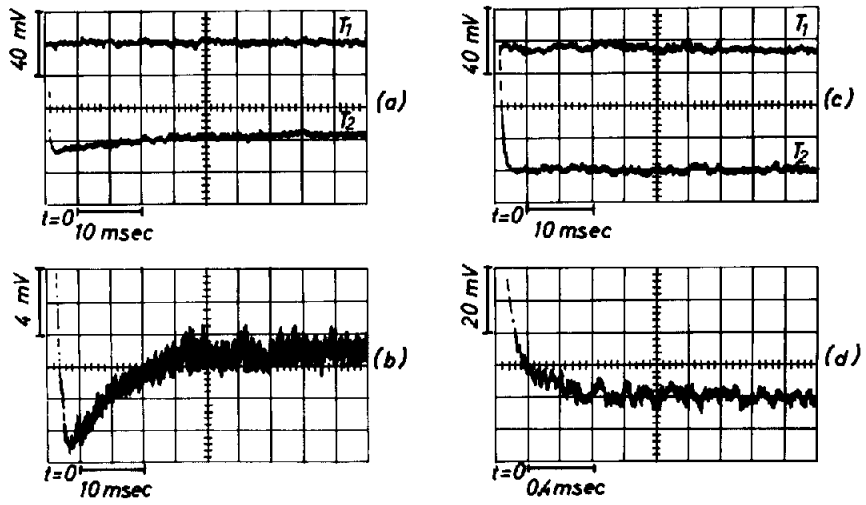

Fig. 3. Oscillograms of fluorescence change after temperature jumps from 24.4 to 27.2°. 1.1 × 10^{-7} M SRS and 6.4 × 10^{-7} M $tRNA^{Ser}$ (a, b) or 1.1 × 10^{-6} M $tRNA^{Phe}$ (c, d).

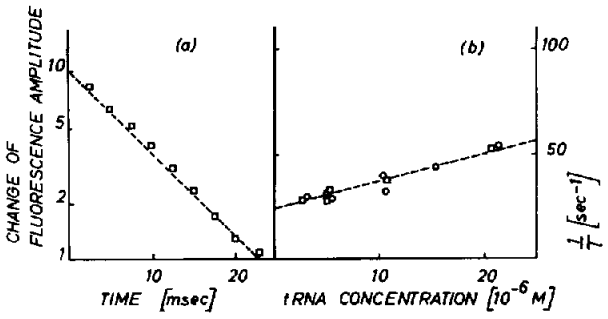

Fig. 4. a) Semilogarithmic plot of fluorescence change. Data from fig. 3b. (b) Concentration dependence of $1/\tau$ for the association of $tRNA^{Ser}$ and SRS. Concn. tRNA ≫ concn. SRS. Values from two experiments.

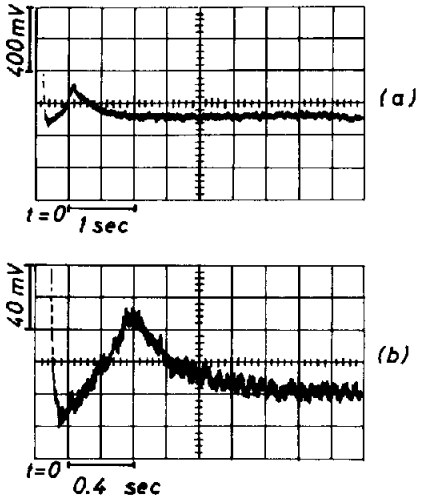

Fig. 5. Oscillograms of fluorescence change after stopped flow mixing at 24.5°. 1.0 × 10^{-7} M SRS and 1.0 × 10^{-7} M $tRNA^{Ser}$ (a) or 1.0 × 10^{-7} M $tRNA^{Phe}$ (b).

interactions a binding process with $n = 0.5$ and $K_{ass} = 8 \times 10^7$ M^{-1} was found. The complexity of the SRS tRNA interactions is evident from the fact that the titration curves at low tRNA concentrations and at higher temperatures could not be readily represented by Scatchard plots.

Temperature jump experiments with SRS $tRNA^{Ser}$ revealed a relaxation process with a small amplitude (max. 1% of the total signal) which was superimposed on a fast quenching process arising from temperature desactivation of fluorescence (fig. 3a, b). A single concentration dependent binding process with the rate constants 1.3 × 10^7 M^{-1} sec^{-1} and 24 sec^{-1} was found corresponding to a K_{ass} of 5.4 × 10^5 M^{-1} (fig. 4a, b). Less pure fractions gave higher values. The analysis of the SRS $tRNA^{Phe}$ interactions (fig. 3c, d) was less accurate because of a low signal/noise ratio. The data indicate a concentration dependent process with rate constants close to 10^9 M^{-1} sec^{-1} and 10^3 sec^{-1}.

The observation of superimposed fluorescence

quenching and enhancing processess (fig. 1) and of positive and negative amplitudes (fig. 3) is best explained by assuming a negative ΔH of both the quenching and the enhacing processes of the SRS/$tRNA^{Ser}$ interaction and the enhancing process of the SRS/$tRNA^{Phe}$ interaction; the quenching process of the latter interaction, however, should have a negligible ΔH, which can be envisaged in molecular terms. In stopped flow experiments a fast and relatively strong fluorescence quenching was followed by slower enhancement and quenching processes (fig. 5a, b). The first quenching must be attributed to the interaction between SRS and the tRNAs, while the other two processes which were not concentration dependent are probably related to conformational changes of the enzyme tRNA complexes. As expected the specific and the unspecific interactions (fig. 5a, b, respectively) differed in the amplitudes.

4. Discussion

From the experiments described in this paper a rather complicated picture of the synthetase tRNA interaction evolves. We are engaged in a more detailed analysis of those processes which were observed up to now and those which have to be searched for. The following interpretations, however, seem to be warranted already at the present state of the work.

The kinetics of the interaction of SRS with both, $tRNA^{Ser}$ and $tRNA^{Phe}$, comprise a fast reaction step with similar K_{ass}; the rate constants, however, are more than an order of magnitude higher in the unspecific interaction compared with the specific one. In the SRS $tRNA^{Val}$ interaction the K_{ass} is much smaller indicating a dissociation rate constant even higher than the one of the SRS $tRNA^{Phe}$ interaction. The forward reaction rate constants may reflect a diffusion controlled binding of the tRNAs to unspecific sites on the enzyme surface and in addition, in the case of the cognate tRNA, to a specific but less accessible site. According to the dissociation rate constants the cognate tRNA remains longer on the enzyme than the other tRNAs as expected in a recognition process. Because of the high rate constants of the unspecific interactions the process of selection of the right tRNA by the synthetase will not be rate limiting. The difference between the observed rate constants and the turnover number of charging of $tRNA^{Ser}$ (10–15 min, [7]) may represent the time interval necessary for the catalytic step.

Acknowledgements

We thank M.Eigen for his catalytic activity at the start of this work. B.Larsson and R.Lamm contributed expert technical assistance. The collaboration between our laboratories was made possible by a grant from Deutsche Forschungsgemeinschaft, SFB 51 for the travel of U.Pachmann. In addition the work was supported by Swedish Medical Research Council, Swedish Cancer Society, and Fonds der Chemischen Industrie.

References

[1] M.Yarus, Ann. Rev. Biochem. 38 (1969) 841.
[2] H.G.Zachau, Angew. Chem. 81 (1969) 645; intern. Edit. 8 (1969) 711.
[3] R.B.Loftfield, in: Protein Synthesis, Vol. I, ed. E. McConkey, in press.
[4] C.Helene, F.Brun and M.Yaniv, Biochem. Biophys. Res. Commun. 37 (1969) 393.
[5] C.J.Bruton and B.S.Hartley, J. Mol. Biol. 52 (1970) 165.
[6] R.Rigler, T.Jovin and C.R.Rabl, to be published.
[7] R.Hirsch, U.Pachmann and H.G.Zachau, manuscript in preparation.
[8] M.H.Makman and G.L.Cantoni, Biochemistry 4 (1965) 1434.
[9] O.H.Lowry, M.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[10] H.G.Zachau, D.Dütting and H.Feldmann, Z. Physiol. Chem. 347 (1966) 212.
[11] P.Philipssen, R.Thiebe, W.Wintermeyer and H.G.Zachau, Biochem. Biophys. Res. Commun. 33 (1968) 922.
[12] M.Ehrenberg and R.Rigler, to be published.
[13] R.Rigler, to be published.