INTERACTIONS BETWEEN AMINOACYL tRNA SYNTHETASES, tRNAs, AND FLUORESCENT DYES

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1. Introduction

The fluorescence of tryptophan residues of the aminoacyl tRNA synthetases has been used to investigate the equilibria [1–3] and kinetics [3] of the binding between synthetases and tRNAs. Further insight into the interactions could be obtained by introducing a dye with a high fluorescence quantum yield which binds preferentially to the tRNA. The binding of ethidium bromide (EB) to 2 tRNAs, various separated and recombined tRNA half molecules, and to seryl tRNA synthetase (SRS) was measured by following its fluorescence intensity and polarization. In the SRS-$tRNA^{Ser}$ interactions, pronounced sigmoid binding curves were observed in the presence of EB which may be related to a dissociation of the enzyme into subunits. The binding of tRNA fragments and fragment combinations to SRS is discussed.

2. Materials and methods

2.1. *Synthetases, tRNAs, and tRNA fragments*

Highly purified SRS and tRNAs from yeast were used as in [3]. The molecular weight of SRS was taken as 120,000 on the basis of disc electrophoresis under dissociating conditions with rabbit muscle pyruvate kinase, catalase, and bovine serum albumin as markers for the SRS monomer [4], in agreement with Heider et al. [5]. Highly purified phenylalanyl tRNA synthetase (PRS) [4] had similar properties as the one described by Fasiolo et al. [6]. 1 A_{280} unit SRS was 0.82 mg according to measurements in a differential refractometer [7]; the determination was based on a specific refractive increment of 0.178 ml/g (with this value refractometric protein determinations with valyl tRNA synthetase gave the same results as obtained [8] with a Kjeldahl procedure); a similar value was obtained by gravimetric determinations [5]. $tRNA^{Ser}$ and $tRNA^{Phe}$ were prepared from brewer's yeast tRNA. Yeast $tRNA^{Val}$ was a gift of U. Lagerkvist. Half molecules of $tRNA^{Ser}$ (Ser 1–34, Ser 36–85) and $tRNA^{Phe}$ (Phe 1–36, Phe 38–76) were prepared by F. Fittler (unpublished) and R. Thiebe [9], respectively, and were kindly donated. In the experiments with fragment combinations equimolar amounts were heated to 70° for 1 min prior to the measurement.

2.2. *Fluorescence titrations and kinetic measurements*

All experiments were performed in 0.03 M K phosphate, pH 7.3, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM GSH, 10% (v/v) glycerol. During all experiments SRS activities remained constant within the error of the test. Titrations were carried out as described previously [3, 10]; In experiments with EB (a gift of Boots Pure Drug Co. Ltd., Nottingham) the fluorescence was excited at 546 nm and measured after passing a Schott GG 590 cutoff filter. The fluorescence decay times of EB were measured in a phase fluorimeter essentially identical to the one of [12], those of tRNA-EB and

tRNA-EB-SRS after excitation at 528 nm by a 6 nsec pulse of a frequency doubled Nd-laser [13]. Temperature jump and stopped flow experiments were performed in the apparatus outlined in [10]. For the mixing a movable mixing chamber [11] equipped with Gibson jets with a dead time of 2 msec was used.

3. Results

In initial experiments EB and proflavine (PF) were compared as fluorescent markers (table 1). EB was selected since the fluorescence of the unbound dye and of the EB-SRS complex could practically be neglected when excitation was at 546 nm. In addition, the comparatively long fluorescence lift time of the tRNA-EB complex is favorable for the measurement of changes in polarization upon binding to the synthetase.

Fig. 1 indicates that $tRNA^{Ser}$ has one strong and about 8 weaker binding sites for EB. The cooperative phase of the Scatchard plot (fig. 1b) is similar to the one observed in the case of Mn^{2+} binding to tRNA [14]. In the case of $tRNA^{Phe}$ strong binding of one EB was found in agreement with [5]. The binding of EB to $tRNA^{Ser}$ fragments and fragment combinations shows the same behavior in the concentration range corresponding to an r up to 1.

When $tRNA^{Ser}$-EB was titrated with SRS (fig. 2b, d), a pronounced S-shape was observed, which was also seen in the corresponding saturation functions (not shown). A similarity shaped curve was found for $tRNA^{Phe}$-EB, although saturation required much higher SRS concentrations (fig. 2d). In most titrations of SRS with $tRNA^{Ser}$, $tRNA^{Phe}$, and $tRNA^{Val}$ the curves had no pronounced S-shape (fig. 2a, c). The values for the binding constants and for the number of tRNA binding sites vary at 5° with the SRS concentrations (3.4 to 7.9×10^{-7} M) from $K = 3 \times 10^7$ to $K = 3 \times 10^8$ M^{-1} and from $n = 1.5$ to $n = 2.0$; at 21° from $K = 1 \times 10^7$ to $K = 3 \times 10^7$ M^{-1} and from $n = 1.2$ to $n = 1.5$. These values were obtained from least square fits of n and K to the experimental points for non-cooperative binding to the dimeric enzyme (drawn lines in fig. 2a, c). Also the specific activity of SRS under assay conditions (as in [3] but at 4X higher tRNA concentrations) was a function of the SRS concentration. For very high concentrations it extrapolated to twice the value obtained under standard conditions; half maximal activity was observed at about 10^{-8} M SRS.

In preliminary titration experiments with PRS and $tRNA^{Phe}$, with and without EB, the curves had no pronounced S-shape.

The decrease in quantum yield in the $tRNA^{Ser}$-EB-SRS complexes is not accompanied by an equivalent decrease of fluorescence life time (table 1). This indicates that part of the EB is displaced from the tRNA by SRS.

Table 1
Fluorescence properties of free and bound dyes at 15°.

	Rel. fluorescence intensity[a]	Rel. quantum yield[b]	Life time of fluorescence (10^{-9} sec)	Degree of polarization[c]
EB	1.0	1.0	1.6	0.064
$tRNA^{Ser}$-EB	37.1	10.3	21.3	0.287 (0.258)
$tRNA^{Phe}$-EB	47.2	10.0[d]	–	0.275[d]
$tRNA^{Ser}$-EB-SRS	28.5	6.2	20.6	0.280
PF		1.0	–	0.014
$tRNA^{Ser}$-PF		0.18	–	0.316 (0.130)
$tRNA^{Phe}$-PF		0.94[d]	–	0.047[d]
$tRNA^{Ser}$-PF-SRS		0.29	–	0.141

[a] Excited at 546 nm, last points of titration curves.
[b] Excited at 507 nm for EB and 455 nm for PF (isosbestic regions).
[c] Values in parentheses were measured at the same tRNA-dye concentrations as in the complexes with SRS.
[d] Values measured at 19°.

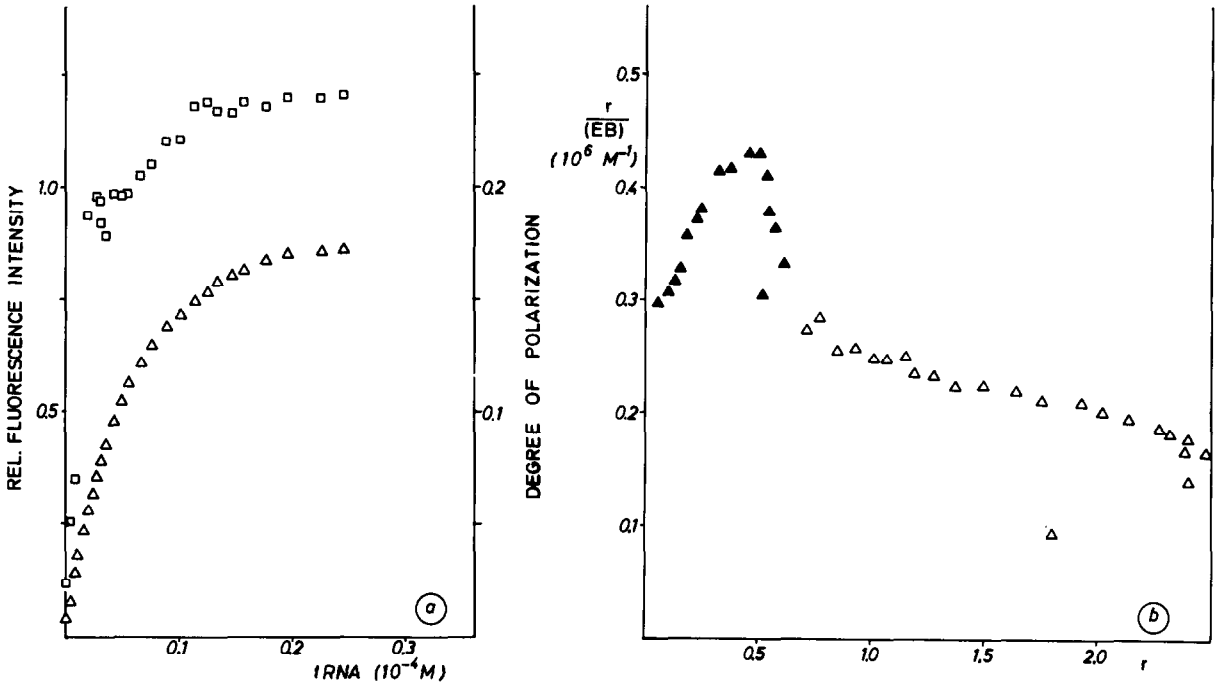

Fig. 1. (a) Fluorescence titration of 2 × 10^{-5} M EB with $tRNA^{Ser}$ at 26°; △ relative intensity, □ degree of polarization. (b) Scatchard plot of the intensity data from a. (△) and an analogous titration curve at 2 × 10^{-6} M EB (▲). r is the number of moles EB bound per mole tRNA. (EB) is the concentration of free EB.

When the EB-complexes of $tRNA^{Ser}$, Ser 36–85, or of fragment combinations containing Ser 36–85, were titrated with SRS, saturation was reached according to the degree of polarization (fig. 3a, b, c). The affinities of the EB complexes of Ser 1–34 and Ser 1–34/Phe 38–76 to SRS were significantly lower (fig. 3b, c). Only with the homologous fragment combination a S-shaped binding curve similar to the one of $tRNA^{Ser}$ was observed. The fluorescence properties of EB were influenced also by SRS alone (fig. 3d), but due to the very low quantum yields of EB and EB-SRS complexes the contribution of EB-SRS fluorescence to the changes in polarization in tRNA-EB-SRS complexes is negligible.

The velocity of aminoacylation of $tRNA^{Ser}$ by SRS was inhibited by less than 10% [4] by the EB concentrations employed in the fluorescence measurements. This control was necessary since inhibition by EB had been observed for a number of aminoacyl tRNA synthetases [16].

Temperature jump experiments observing the polarized fluorescence of $tRNA^{Ser}$-EB-SRS complexes indicate the presence of at least two relaxation times in the millisecond to second range which can be attributed to the interaction of $tRNA^{Ser}$-EB with SRS. Preliminary stopped flow experiments with the complexes show a recombination time of 200 msec, while the slow effects in $tRNA^{Ser}$-SRS combinations without EB do not seem to be reproducible under the conditions of fig. 5 in [3].

4. Discussion

When SRS at a relatively high concentration was titrated at 5° with $tRNA^{Ser}$, two molecules of tRNA were bound to the enzyme (fig. 2a), in agreement with previous measurements [3]. Due to the improved method of correction [10] the present values for the binding constants are more accurate than the previous ones [3].

In dilute SRS solutions the binding constant, the

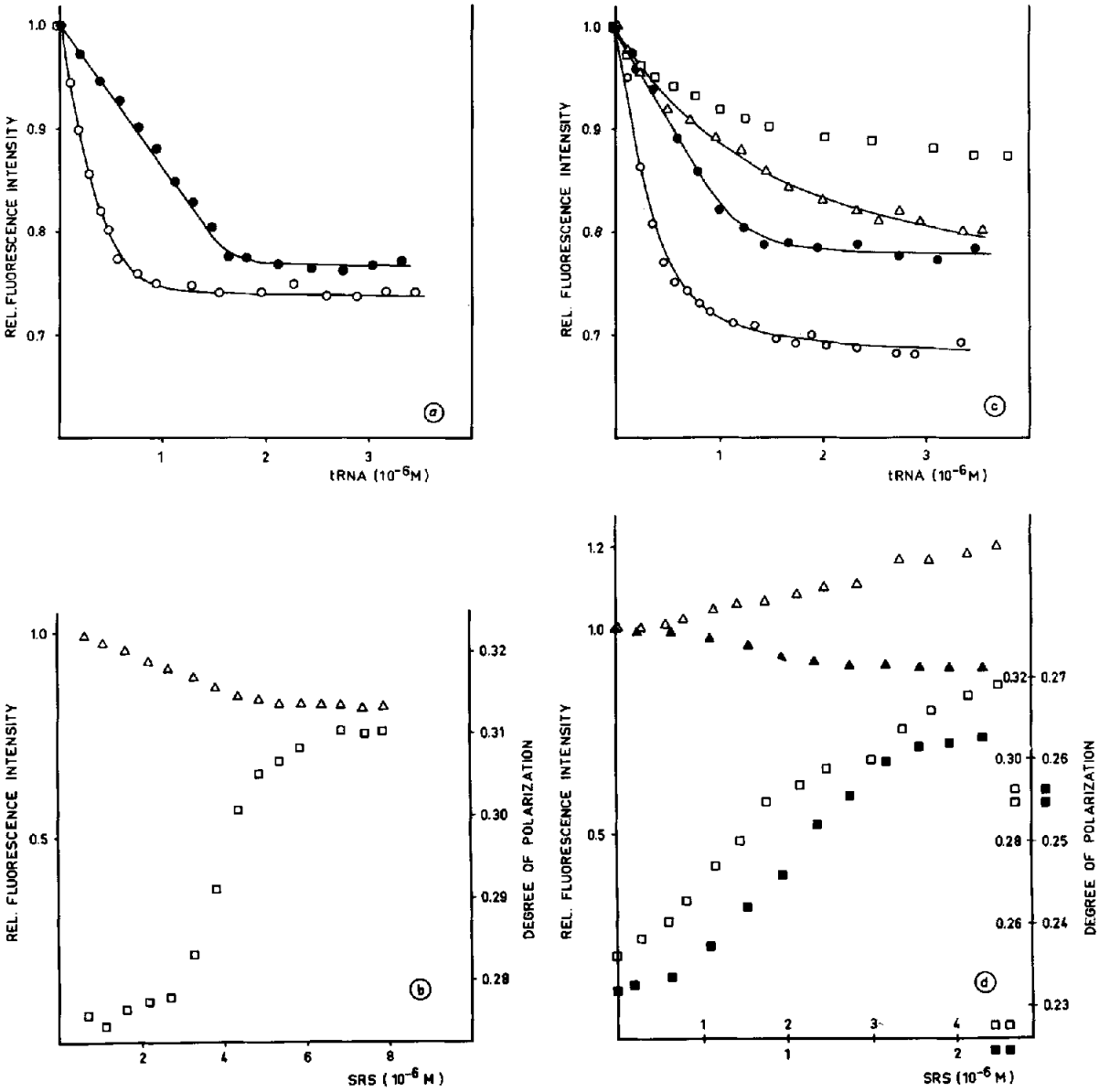

Fig. 2. (a) Fluorescence titration of 3.4 and 7.9 × 10^{-7} M SRS (○ and ●, respectively) with $tRNA^{Ser}$ at 5°. SRS concentrations were kept constant during the whole experiments. Drawn lines are calculated curves, see text. (b) Fluorescence titration of $tRNA^{Ser}$-EB complex with SRS at 5°. $tRNA^{Ser}$ and EB concentrations were kept at 1.0 × 10^{-6} M. Symbols as in fig. 1a. (c) Titrations at 21°, otherwise as in a. Titration of 3.4 × 10^{-7} M SRS with $tRNA^{Ser}$ (○), $tRNA^{Phe}$ (△), and $tRNA^{Val}$ (□); 7.9 × 10^{-7} M SRS with $tRNA^{Ser}$ (●). (d) Titrations of $tRNA^{Ser}$-EB (1 × 10^{-6} M, full symbols) and $tRNA^{Phe}$-EB (2.1 × 10^{-6} M, open symbols) with SRS at 21°. Relative intensity (△, ▲); degree of polarization (□, ■).

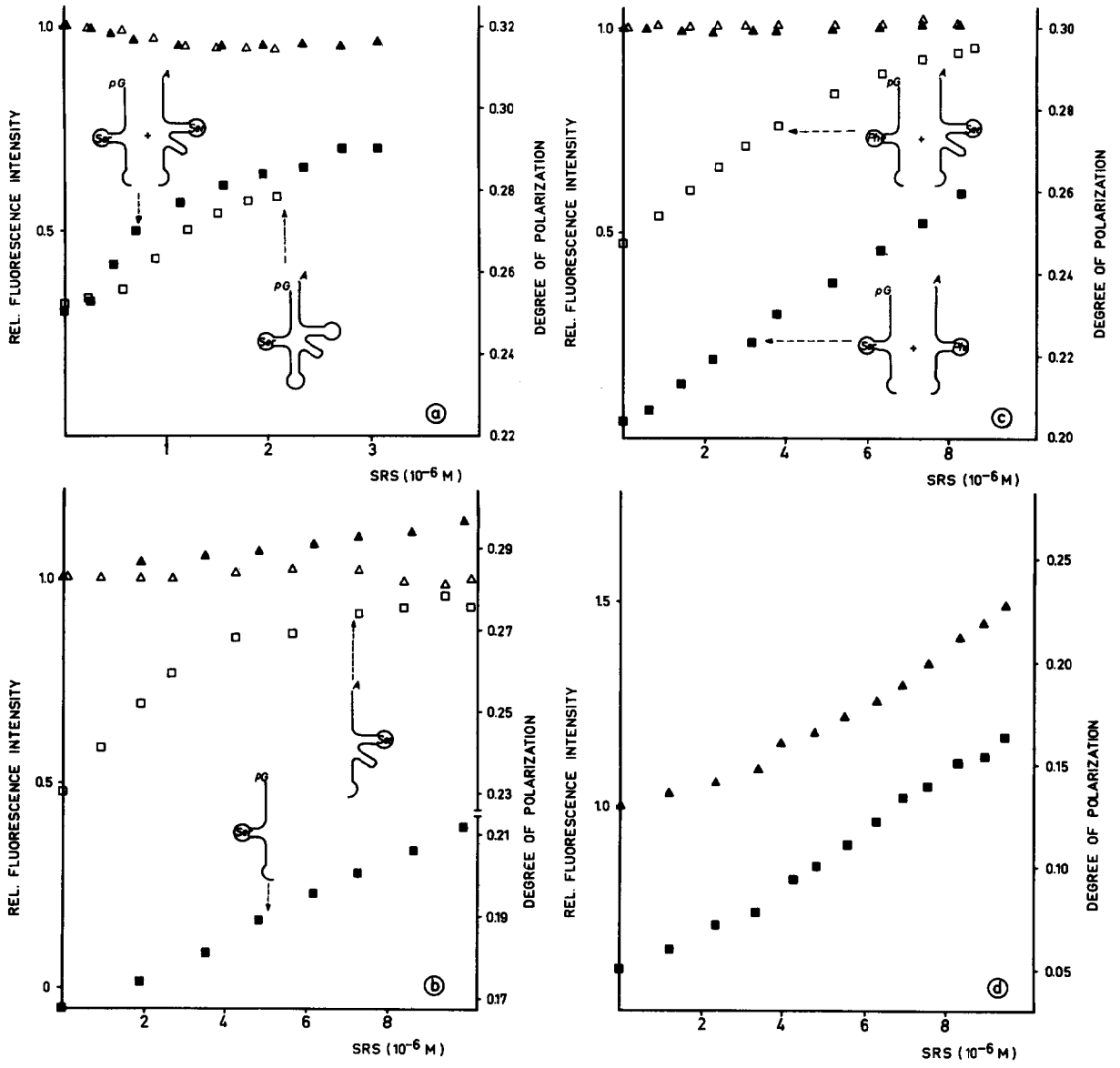

Fig. 3. Fluorescence titrations with SRS at 14° of (a) $tRNA^{Ser}$-EB (open symbols), Ser 1–34/Ser 36–85–EB (full symbols); (b) Ser 1–34–EB (full symbols), Ser 36–85–EB (open symbols); (c) 1–36/Ser 36–85–EB (open symbols), Ser 1–34/Phe 38–76–EB (full symbols); (d) EB. EB and tRNA or fragment concentrations during the whole experiment were kept at 2×10^{-6} M (a) or 3×10^{-6} M (b–d). (△, ▲) relative intensity, (□, ■) degree of polarization.

apparent n, and also the specific activity were lower than in concentrated ones. This indicates that SRS either dissociates into subunits and/or changes its conformation. The apparent molecular weight of SRS decreases on dilution according to ultracentrifuge studies of Heider et al. [5] and of ourselves.

When $tRNA^{Ser}$-EB was titrated with SRS a cooperative binding behavior was observed (fig. 2b, d). Simulations of the saturation functions have been calculated which show that such behavior can result from a dissociation into subunits with a changed affinity to tRNA. Kinetic studies will make it possible to distinguish whether the cooperative behavior is due to a dissociation of SRS or to a conformational change of the undissociated enzyme. The strong fluorescence and polarization of the tRNA-EB complexes apparently allow the registration of more intermediate steps than the tryptophan fluorescence of the SRS [3]. When $tRNA^{Phe}$ binds to SRS at 21° the number of binding sites ($n = 2$) and the binding constant ($K = 1 \times 10^6\ M^{-1}$) are nearly independent of enzyme concentration which indicates that SRS selects $tRNA^{Ser}$ better in the dimeric state. This is supported by the finding that at higher salt concentrations, under which probably the dimeric state is favored, SRS does not bind $tRNA^{Phe}$ while $tRNA^{Ser}$ binding is complete.

The finding that the combination of $tRNA^{Ser}$ half molecules is bound to SRS, although with a somewhat lower affinity than $tRNA^{Ser}$, was expected since binding had also been observed in sucrose gradient centrifugations [4], and this combination can also be enzymatically charged with serine (F. Fittler, unpublsihed). The observed binding of the heterologous combinations of half molecules, on the other hand, is interesting with respect to the as yet unsuccessful attempts of charging these combinations with amino acids (F. Fittler, unpublsihed). It may be noteworthy that Ser 36–85 and fragment combinations containing this half molecule have a higher affinity to SRS than Ser 1–34 and its combination with Phe 38–76. This may indicate that parts of the CCA-half molecule (Ser 36–85), as the miniloop region, are important for the $tRNA^{Ser}$ binding to SRS and/or the recognition process. The mechanism of binding of the homologous fragment combinations to SRS seems to be more similar to the mechanism of $tRNA^{Ser}$ binding than the one of the heterologous combinations since only the titration curve of the former shows a cooperative behavior.

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