FLUORESCENCE STUDIES OF THE BINDING OF VALYL-tRNA SYNTHETASE AND TRYPTAMINE TO VALINE-SPECIFIC tRNA. A POSSIBLE ROLE FOR TRYPTOPHAN RESIDUES IN THE BINDING OF AMINOACYL-tRNA SYNTHETASES TO tRNAs

Claude HELENE

Centre de Biophysique Moléculaire, 45-Orleans-02, France

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1. Introduction

Many studies dealing with the specific binding of aminoacyl-tRNA synthetases to their cognate tRNAs have been devoted to the determination of the tRNA recognition site [1, 2]. Little is known about the aminoacid residues of synthetases involved in the binding of their substrates. Specific labelling of the aminoacid binding site of the enzyme has been reported for the methionine system from *E. coli* and the sequence of the labelled peptide has been determined [3]. A few sulfhydryl groups do not react with specific reagents in the presence of the aminoacyl adenylate but this does not prove that they are involved in binding [4, 5]. Protonation of aminoacid residue(s) with a pK around 6 (probably histidine) increases the affinity of the synthetase for tRNA [6, 7]. But this could be a long-range effect due to a conformational change of the enzyme molecule. We reported previously that the fluorescence of tryptophan residues of valyl-tRNA synthetase from *E. coli* ($S^{val.}_{E.coli}$) is quenched (about 20%) upon binding of valine-specific tRNA [6, 8, 9]. Different mechanisms have been proposed to explain this quenching [9]. Similar quenching effects have also been observed in other systems [3, 10, 11]. We wish to report here the results of fluorescence decay measurements on the system $S^{val.}_{E.coli}$ – $tRNA^{val}$ as well as fluorescence studies of the binding of tryptamine and 5-hydroxytryptamine (serotonin) to tRNA.

These experiments strongly suggest that quenching of the synthetase fluorescence upon binding tRNA could be due to a direct interaction between one or two tryptophan residues of the enzyme and bases of the tRNA molecule. These tryptophan residues could intercalate between bases thus maintaining the tRNA molecule in a definite position with respect to the enzyme molecule.

2. Experimental

Valyl-tRNA synthetase and $tRNA^{val}_1$ from *E. coli* were gifts from Dr. M. Yaniv and purified as previously described [5]. Total *E. coli* tRNA was purchased from Schwarz Bioresearch. Tryptamine and serotonin were obtained from Calbiochem.

Interactions between the synthetase and tRNA were investigated in buffer A: sodium cacodylate 0.03 M, $MgCl_2$ 0.006 M, pH 5.5. The binding of tryptamine and serotonin to tRNA was studied in buffer B: sodium cacodylate 1 mM, NaCl 1 mM, pH 7.

Fluorescence measurements were performed with a Jobin-Yvon spectrofluorometer [6]. The sample was contained in a 1 cm quartz cell. The fluorescence intensity of each sample was compared to that of a reference solution usually containing 10^{-5} M tryptamine or serotonin. To take into account the screening effect of tRNA at the excitation wavelength, a theoretical relationship [9, 10, 12] may be used under our experimental conditions, when the absorbance is less than 0.2 (geometrical factors prevent using this relationship at higher absorbances). In most

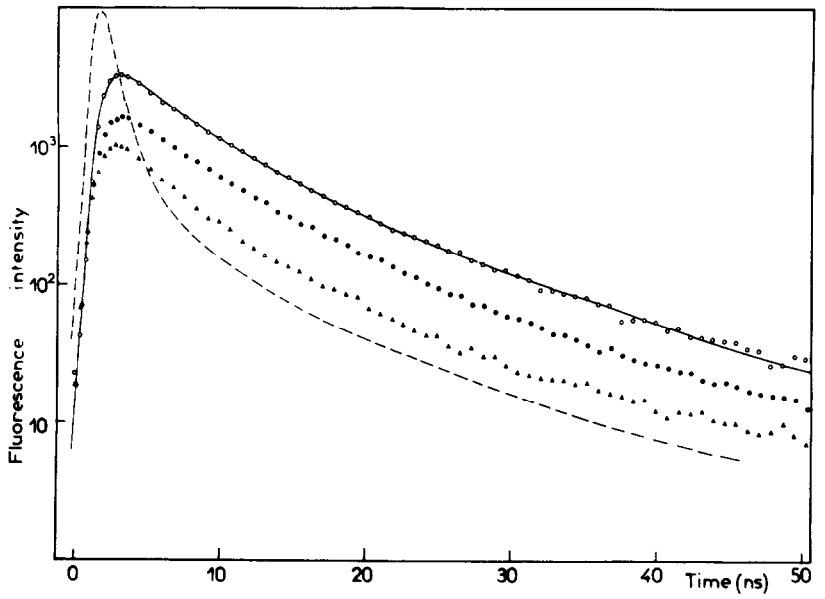

Fig. 1. Fluorescence decay (semi-logarithmic plot) of 3×10^{-7} M valyl-tRNA synthetase alone (○), in the presence of 4.8×10^{-6} M $tRNA_1^{val}$ (●) and in the presence of 10^{-5} Cu^{2+} (△). All measurements in buffer A. The full line was calculated as a superposition of two exponential decays with lifetimes 3.8 and 12 nsec and respective contributions 85% and 15% (see text).

cases, an empirical correction curve was experimentally obtained under given experimental conditions (slit widths, excitation and emission wavelengths, cell position) by measuring the apparent fluorescence quantum yield of a tryptamine or serotonin solution in the presence of increasing amounts of a mixture of nucleosides (interactions between tryptamine or serotonin and nucleosides [13, 14] are too weak to give any measurable quenching in the concentration range investigated). In the studies of tryptamine or serotonin binding to tRNA, the relative overall fluorescence quantum yield of bound and free molecules was determined by measuring the fluorescence intensity in buffer B before and after adding 1 M NaCl, respectively. Under the latter conditions, the complex can be practically considered as fully dissociated (see below).

Fluorescence decay measurements under flash excitation were performed with an apparatus described by Wahl [15] using the single photon counting technique.

3. Results and discussion

3.1. *Quenching of tryptophan fluorescence of $S^{val}_{E.coli}$ upon binding $tRNA^{val}$*

As reported earlier [8, 9], aminoacyl-tRNA synthetases usually contain tryptophan residues whose fluorescence can be used to follow the binding of tRNAs. When increasing amounts of $tRNA^{val}$ are added to a solution of $S^{val}_{E.coli}$ in buffer A, the fluorescence quantum yield of the enzyme decreases until a plateau is reached which corresponds to a quenching of about 20%. Extrapolation of the first part of the quenching curve indicates that a 1:1 complex is formed [8, 9]. When increasing amounts of the synthetase are added to a $tRNA^{val}$ solution, similar results are obtained (unpublished results).

Different hypotheses can be put forward to explain the quenching of tryptophan fluorescence upon binding of tRNA to the synthetase. They have been discussed previously [9]. To obtain more information, we measured the decay of $S^{val}_{E.coli}$ fluorescence under flash excitation. The decay is not exponential as expected since the enzyme contains about 11 tryptophan residues (and it is not known

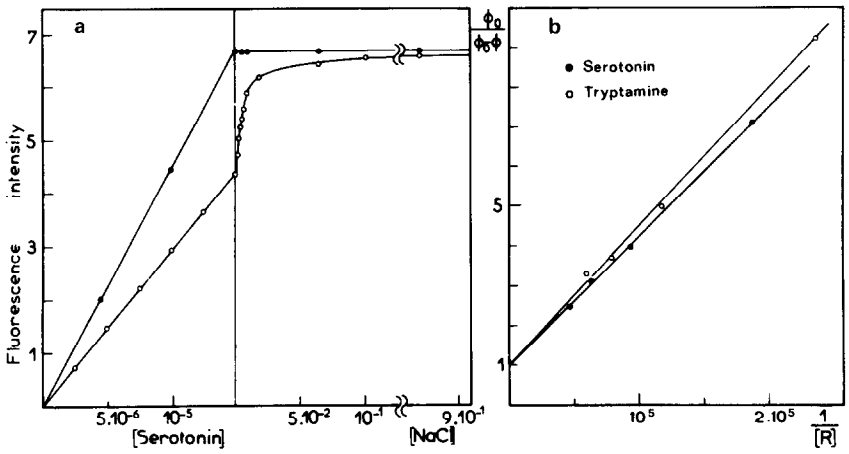

Fig. 2. (a) *Left part*: concentration dependence of fluorescence intensity for serotonin in the absence (•) and the presence (○) of 2×10^{-5} M $tRNA_1^{val}$ in buffer B at 16°. Excitation wavelength: 305 nm. Fluorescence intensity measured at 345 nm (corrected for the screening effect of tRNA). *Right part*: Change of fluorescence intensity with sodium chloride concentration (corrected for dilution and changes in screening effects). (b) Analysis of fluorescence quenching by $tRNA_1^{val}$ according to equation (1) (see text). [*R*] is the concentration in tRNA.

whether all of them are fluorescent). However, the decay curve can be fitted by a sum of two exponentials whose corresponding lifetimes are 3.8 nsec and 12 nsec (fig. 1). Their relative contributions are 85% and 15% respectively. The fluorescence quantum yields and lifetimes of tryptophan residues in proteins depend very much upon their environment. The fluorescence lifetime of the model compound *N*-acetyl-L-tryptophan amide is about 3.8 nsec in aqueous solutions at pH 7. Thus most of the fluorescent tryptophan residues of $S_{E.coli}^{val}$ appear to be accessible to the solvent and are thus on the outside of the enzyme molecule. This is in agreement with the position of the fluorescence maximum (345 nm). The 12 nsec lifetime would correspond to tryptophan residues buried inside the enzyme structure and having a fluorescence quantum yield about three times higher than the outside residues.

The decay of $S_{E.coli}^{val}$ fluorescence is not markedly affected by the binding of $tRNA^{val}$ (fig. 1). The experimental decay curve can be fitted by a sum of two exponentials whose corresponding lifetimes are identical with those of the free enzyme. Although this superposition of two exponential decays is probably not a unique solution, a comparison of the decay curves for the free and bound enzymes shows that there is no evidence at short times for any contribution of residues with short lifetime. We have observed that Cu^{2+} ions bind to the synthetase and quench its fluorescence. In this case, a shortening of the fluorescence lifetime of some tryptophan residues can be clearly seen (fig. 1).

The absence of any observable change in the fluorescence decay of $S_{E.coli}^{val}$ upon binding tRNA suggests that the observed 20% quenching of the fluorescence quantum yield could be ascribed to a complete quenching of one or two tryptophan residues of the enzyme. This could be due to a change in the enzyme conformation which makes possible an interaction of these residues with quenching groups inside the enzyme structure. However, we have previously shown that tryptophan and its derivatives interact with nucleic acid bases and this interaction leads to a complete quenching of their fluorescence [13]. Thus, the quenching of one or two tryptophan residues on the outside of the enzyme could be ascribed to a direct interaction of these residues with bases of the tRNA molecule. Experiments showing that tryptamine and 5-hydroxytryptamine (serotonin) can bind to tRNA and that the fluorescence of bound molecules is quenched provide supporting evidence to these hypotheses. They are described below.

3.2. *Binding of tryptamine and 5-hydroxytryptamine (serotonin) to tRNAs*

We already reported that the fluorescence of tryptamine and serotonin is quenched in the presence of DNA [16]. Fluorescence lifetime measurements and quenching curve analysis have demonstrated that fluorescence quenching is due to an interaction of the indole ring with the purine and pyrimidine bases. The fluorescence of tryptamine and serotonin is also quenched upon addition of total tRNA or valine specific tRNA from *E. coli* (fig. 2).

The change in the overall apparent fluorescence quantum yield (ϕ) with tRNA concentration (R) can be analyzed according to the following relationship which can be used when the concentration of the fluorescent amine is low as compared to that of binding sites [16]:

$$\frac{\varphi_0}{\phi_0 - \phi} = \frac{\phi_0}{\phi_0 - \phi_b} + \frac{\varphi_0}{\phi_0 - \phi_b} \frac{1}{K_n} \frac{1}{[R]} \qquad (1)$$

where ϕ_0 and φ_b are the fluorescence quantum yields of free and bound tryptamine (or serotonin) molecules, respectively. K is the binding constant and n the number of binding sites per tRNA molecule.

Plots of $\phi_0/(\phi_0 - \phi_0)$ against $1/[R]$ are straight lines (fig. 2) whose y-axis intercept $\phi_0/(\phi_0 - \phi_0)$ is equal to 1 indicating that $\phi_b \simeq 0$. This means that bound tryptamine or serotonin molecules do not fluoresce at all as already observed with DNA [16]. Fluorescence quenching seems to be related to charge-transfer interactions in stacked complexes [13, 14, 16]. The values of K_n obtained according to eq. (1) are 2.85×10^4 M^{-1} and 3.15×10^4 M^{-1} for tryptamine and serotonin, respectively. They are quite similar to those obtained with double stranded DNA [16] (if calculated per tRNA phosphate).

As already reported in the case of DNA, the binding of tryptamine and serotonin is strongly dependent upon sodium chloride concentration (fig. 2). This is ascribed to a competition between Na^+ ions and positively charged NH_3^+ groups of the aromatic amine toward the negatively charged phosphate groups of the nucleic acid. Complex dissociation at high ionic strength is accompanied by an increase of the fluorescence quantum yield (fig. 2).

4. Conclusion

The quenching of the fluorescence emitted by tryptophan residues of aminoacyl-tRNA synthetases upon binding to their cognate tRNAs has been observed in several cases. The fluorescence decay measurements reported above suggest that, in the case of valyl-tRNA synthetase from *E. coli*, one or two tryptophan residues are completely quenched. Tryptamine and 5-hydroxytryptamine (serotonin) can bind to total tRNAs and to valine-specific tRNA from *E. coli*. This binding involves a direct interaction between the indole ring and the purine and pyrimidine bases of the tRNA molecule [13, 14, 16] which leads to fluorescence quenching. These results support the hypothesis that one or two tryptophan residues of the synthetase could interact with the tRNA bases for example by intercalation between stacked bases in single-stranded or double-stranded regions. This could help maintaining the tRNA molecule in a definite position with respect to the enzyme molecule thus allowing further reactions to take place.

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