FLUORESCENCE STUDY OF INTERACTIONS BETWEEN VALYL-tRNA SYNTHETASE AND VALINE-SPECIFIC tRNAs FROM ESCHERICHIA COLI

Claude Hélène and Francine Brun
Centre de Biophysique Moléculaire, La Source, 45 - Orléans

Moshe Yaniv
Service de Physiologie Microbienne, Institut de Biologie Physico-Chimique, Paris (5è)

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Summary

Substrate-induced fluorescence variations have been used to study the association of valyl-tRNA synthetase (VRS) and $tRNA^{val}$. The binding of tRNA to the enzyme leads to a decrease of the enzyme fluorescence quantum yield. A ten-fold increase in the binding constant was observed when the pH was lowered from 7.0 to 5.5. The fluorescence quantum yield of VRS alone decreases when the pH is lowered; a pK of 5.9 is obtained from the fluorescence titration curve. This suggests that ionization of residues with a pK of 5.9 (probably histidine) leads to an increase in the affinity of VRS for tRNA. It is also shown that two different species of $tRNA^{val}$ from E. coli differ in their affinities for the enzyme by a factor of ten.

One of the essential steps in the biosynthesis of polypeptide chains involves the attachment of each amino-acid to its specific tRNA. This selective attachment is realized by a specific enzyme (aminoacyl-tRNA synthetase) which must recognize both the amino-acid and the tRNA. Until now, the experimental methods used to study the interactions between aminoacyl-tRNA synthetases and tRNAs were based upon the separation of the complex from free reactants (Lagerkvist et al, 1966; Yarus and Berg, 1967; Yaniv and Gros, 1969). Among physical methods which can be used to measure equilibrium concentrations, fluorescence spectroscopy seems to be very convenient. Enzymes usually contain aromatic amino-acid residues which emit fluorescence when excited in their ultra-violet absorption band. The formation of an enzyme-substrate complex may affect both spectrum and intensity of the enzyme fluorescence. These modifications can result either from specific interactions between the substrate and some of the fluorescent amino-acid residues or from substrate-induced conformation change of the enzyme molécule (which may modify the environment of the fluorescent chromophores). If one of these conditions is fulfilled, complex formation between an enzyme and its substrate(s) can be conveniently studied by fluorescence spectroscopy (Edelhoch and Steiner, 1964).

Valyl-tRNA synthetase (VRS) contains a number of tyrosine (~ 39) and tryptophan (~ 11) residues (Yaniv and Gros, 1969) and has a high fluorescence quantum yield. We report here the results of a fluorescence study of complex formation between VRS and transfer ribonucleic acids specific for valine ($tRNA^{val}$). Equilibrium constants deduced from these fluorescence measurements are in good agreement with those obtained by membrane binding or sucrose gradient centrifugation (Yaniv and Gros, 1969).

MATERIALS AND METHODS

$tRNA^{val}_1$ and $tRNA^{val}_2$ were separated by fractionation on a benzoylated DEAE-cellulose column (Gillam et al. 1967). These tRNAs were further purified on a second benzoylated DEAE-cellulose column after acylation with pure enzyme and phenoxyacetylation of the free α-amino group and the degree of purity of the preparations obtained exceeded 90%. The sequence of $tRNA^{val}_1$ was established by Yaniv and Barrell (1969). It is the major species (80%) which is coded by GUA and GUG. $tRNA^{val}_2$ (20%) is probably a mixture of two subspecies that differ in a small number of bases outside the anticodon region (Yaniv and Barrell, unpublished results) and it is coded by GUU and GUC. Valyl-tRNA synthetase was purified as described by Yaniv and Gros (1969). The preparation used in this study was at least 95% pure with a specific activity of 400 units/mg (pyrophosphate exchange assay). Fluorescence measurements were carried out with a Jobin-Yvon spectrofluorimeter. The solution was contained in a 1-cm quartz cell and the optical density of VRS was always less than 0.1 at 280 nm. The excitation wavelength was chosen at 290 nm and the fluorescence intensity measured at 350 nm. To take into account fluctuations in lamp intensity, the fluorescence intensity of the sample was compared to that of a reference solution which contained either tryptophan or pure VRS. Fluorescence intensities were also corrected for dilution of VRS due to addition of the substrate, and for absorption of light by tRNA at 290 nm. If d_0 represents the optical density of VRS alone at 290 nm and d the total optical density at 290 nm after addition of tRNA, the true fluorescence intensity I_F is related to the measured intensity I_M by the relation :

$$I_F = I_M \frac{d_0}{d} (1 - 10^{-d})$$

For quantum yield measurements, tryptophan was chosen as a standard. In the conditions used here, the fluorescence spectra of tryptophan alone and of VRS were quite similar. Thus, the quantum yield was determined by direct comparison of the fluorescence intensities at the peak maximum of two solutions containing VRS and tryptophan of the same optical density at the excitation wavelength (290 nm). The quantum yield of tryptophan was assumed to be 0.12 at pH 7 (Böttresen, 1967).

RESULTS AND DISCUSSION

The fluorescence spectrum of VRS excited at 290 nm is similar to that of tryptophan alone in the same conditions ($\lambda max \simeq 345$ nm). We were not able to see any fluorescence from tyrosine. The fluorescence quantum yield decreases when the pH decreases as reported in figure 1. To show that this decrease was not due to a change in the degree of ionization of the buffer, we have performed the same measurements in phosphate and cacodylate buffers. In each case, the same fluorescence titration curve is obtained which allows us to determine a pK value of 5.9. This pK shifts to 5.6 if Mg^{2+} ions are not present in the solution. Shinitzky and Goldman (1967) have already observed that protonated histidine residues quench the fluorescence of tryptophan by charge-transfer complex formation in model oligopeptides. This leads to fluorometric titration curves similar to those reported in figure 1 with pK between 5.5 and 8. VRS contains 5 histidine and 11 tryptophan residues. The change in fluorescence quantum yield with pH is relatively high (figure 1). It seems therefore more likely that ionization of one (or more) residue with a pK of 5.9 (probably histidine) induces a conformation change in VRS and thus modifies the environment, and consequently the fluorescence quantum yield, of several tryptophan residues.

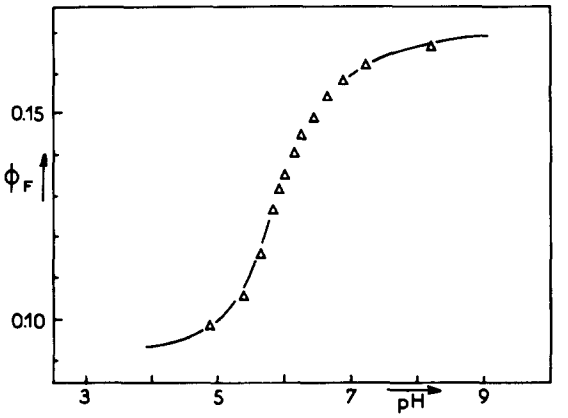

Figure 1 - Variation of valyl-tRNA synthetase fluorescence quantum yield with pH in cacodylate buffer 3×10^{-2}M $MgCl_2$ 6×10^{-3}M

Addition of $tRNA^{val}_1$ to VRS leads to a decrease of the fluorescence intensity of the latter until a plateau is reached (figure 2). Addition of the same tRNA to isoleucyl-tRNA synthetase does not change the fluorescence quantum yield of this enzyme. The variation of VRS fluorescence intensity as a function of tRNA concentration allows us to calculate the equilibrium constant K for the complex formation reaction.

$$VRS + tRNA \rightleftharpoons VRS - tRNA$$

The value of K varies with pH and, as shown in Figure 2 and table 1, K is higher at low pH values. These results are in good agreement with those obtained by other methods on the same system (Yaniv and Gros, 1969). The increase in K seems to be correlated with the decrease in the fluorescence quantum yield of VRS when pH decreases (figure 1). This suggests that the "acid form" of the enzyme binds tRNA more strongly. The fluorescence binding curves are also in agreement with the presence of only one binding site for tRNA per molecule of enzyme (Mw ≃ 110,000). At a given pH, the equilibrium constant K depends on the species of tRNA specific for valine. At pH 6.5, the affinity of $tRNA^{val}_2$ is higher than that of $tRNA^{val}_1$. The equilibrium constants for these two species are 5×10^7 and $0.7 \times 10^7 M^{-1}$ respectively. This higher affinity of $tRNA^{val}_2$ for VRS as compared to $tRNA^{val}_1$ can also be demonstrated by competition experiments. $tRNA^{val}_2$ easily replaces $tRNA^{val}_1$ from the complex VRS-tRNA. VRS saturation curves at pH 5.5 and 6.0 measured by the membrane binding assay developed by Yarus and Berg (1967) also show a higher affinity for $tRNA^{val}_2$ (Helene and Yaniv, manuscript in preparation). Preliminary sequence studies of $tRNA^{val}_2$ suggest that it differs from that of $tRNA^{val}_1$ in the three loops and in three of the four ordered regions. Recently Lagerkvist and Rymo (1969) observed a difference in the affinities of two yeast $tRNA^{val}$ for the yeast synthetase.

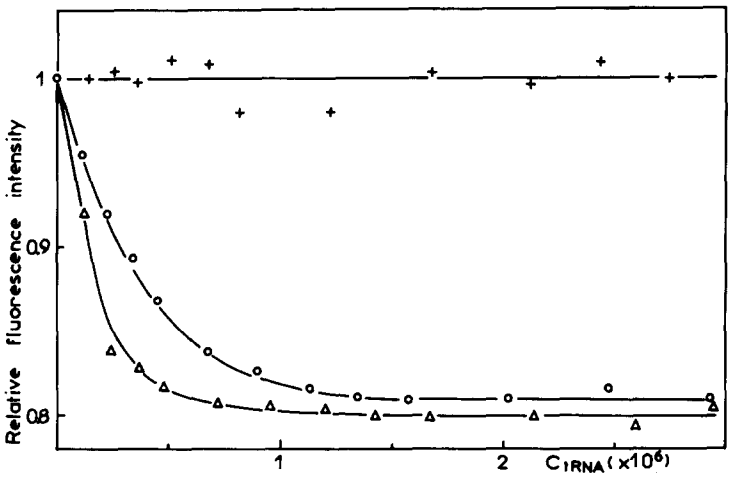

Figure 2 - Relative variation of VRS fluorescence quantum yield as a function of $tRNA^{val}_1$ concentration at pH 5.5 (Δ), and 6.5 (o) in phosphate buffers (enzyme concentration ~ 3×10^{-7}M). (+) fluorescence quantum yield of isoleucyl-tRNA synthetase as a function of $tRNA^{val}_1$ concentration. (enzyme concentration ~ 4×10^{-7}M).

TABLE 1

Equilibrium constants for binding of valine-specific tRNAs to valyl-tRNA synthetase

	Buffer*	pH	$K(M^{-1})$
$tRNA_1^{val}$	cacodylate	7	$5x10^6$
	phosphate	6.5	$7x10^6$
	phosphate	6	$2x10^7$
	phosphate	5.5	$5x10^7$
$tRNA_2^{val}$	phosphate	6.5	$5x10^7$
	phosphate	5.5	$> 10^8$

* the solution contained cacodylate or phosphate buffer $3x10^{-2}M$ and $MgCl_2$ $6x10^{-3}M$. K was estimated from the value of c of tRNA concentration which gives 50% of the maximum decrease of fluorescence intensity : $K \simeq \frac{2}{2c-c_o}$ where c_o equals the initial concentration of VRS. The fluorescence quenching curve was then calculated for this and slightly different values of K until the best fit with the experimental curve was obtained.

The decrease of fluorescence intensity of VRS upon binding of tRNA can be due either to interactions between groups of the tRNA and tryptophan residues of the enzyme or to a change in the environment of the fluorescent chromophores due, for example, to a conformational change in VRS. Such a conformational change has been already observed for tyrosyl-tRNA synthetase by circular dichroism measurements (Ohta et al., 1967). This technique did not reveal any conformational change in VRS upon binding of tRNA (A. Favre, unpublished results). However the technique is not sensitive enough to exclude small local conformational change in the protein. Charge-transfer complexes between tryptophan and nucleosides have been detected by fluorescence in frozen aqueous solutions (Montenay-Garestier and Hélène, 1968). They have very low fluorescence quantum yields when compared to tryptophan. The formation of such complexes could explain the results observed here.

The results presented here show that fluorescence can be a convenient method to study interactions between two large molecules. This methods permits direct measurements of equilibrium concentrations of free and bound macromolecules. Binding of other substrates (Mg^{2+}, valine, ATP) to valyl-tRNA synthetase

as well as interactions between sites for different substrates can also be followed by fluorescence measurements.

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