Neutron Scattering Study of the Binding of $tRNA^{Phe}$ to *Escherichia coli* Phenylalanyl-tRNA Synthetase†

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ABSTRACT: *Escherichia coli* phenylalanyl-tRNA synthetase has been characterized by small-angle neutron scattering. In solution (20 mM imidazole hydrochloride, pH 7.6, 10 mM 2-mercaptoethanol, and 0.1 mM ethylenediaminetetraacetic acid), this enzyme has a molecular weight of 227K ± 20K with a radius of gyration of 48.3 ± 0.6 Å, independent of the presence of $MgCl_2$ up to 50 mM. The change of the scattering upon adding $tRNA^{Phe}$ to the enzyme has been followed with 10 mM $MgCl_2$ present in the buffer. One enzyme molecule is capable of binding two $tRNA^{Phe}$ molecules with affinity constants larger than 10^6 M^{-1}. Parallel titration experiments in 73% ^2H_2O, close to the matching point of tRNA, show that the R_G of the enzyme is not changed by the binding of one or two $tRNA^{Phe}$ molecules. These results are compared with quasi-electric light scattering studies [Holler, E., Wang, C. C., & Ford, N. C., Jr. (1981) *Biochemistry 20*, 861–867] where the addition of either $MgCl_2$ or $tRNA^{Phe}$ was shown to cause dramatic changes of the apparent translational diffusion constant of phenylalanyl-tRNA synthetase.

Aminoacyl-tRNA synthetases are essential enzymes in specifying the fidelity of mRNA translation [for a review, see Schimmel & Söll (1979)]. An unresolved question consists in knowing whether conformational changes are involved in the step of tRNA binding to their cognate aminoacyl-tRNA synthetase. Depending on the used technique, changes in the tRNA structure upon attaching the enzyme have been postulated (Willick & Kay, 1976; Favre et al., 1979) or excluded (Shulmann et al., 1974). In a few cases, kinetic studies have revealed a fast rearrangement of the enzyme/tRNA complex (Riesner et al., 1976; Krauss et al., 1976; Rigler et al., 1976). This rearrangement would depend on the chemical nature of the 3′-terminal end of the tRNA (Krauss et al., 1977, 1979). As to the enzyme, the recent introduction of the small-angle neutron scattering technique has permitted to gain information on the rearrangement of a synthetase in its complex with tRNA (Dessen et al., 1978, 1982; Zaccaï et al., 1979). This technique offers the unique advantage that the scattering density of the solvent can be adjusted to equal that of tRNA by varying the ^2H_2O to ^1H_2O ratio (Jacrot, 1976). Under this condition, the structural parameters of the enzyme alone can be followed as a function of tRNA binding.

Recently, the laser light scattering technique has been employed by Holler et al. (1981) to measure the translation diffusion constant of *Escherichia coli* phenylalanyl-tRNA synthetase and its complexes with Mg^{2+} and cognate $tRNA^{Phe}$. This study showed the synthetase, an $\alpha_2\beta_2$ tetrameric enzyme (Fayat et al., 1974), as an elongated molecule with the remarkable axial ratio of 10 (by estimating a hydration shell of 50% of the volume of the protein). Upon addition of 10 mM $MgCl_2$, the axial ratio increased to 17, indicating an even more elongated form. Moreover, the addition of $tRNA^{Phe}$ to a solution containing enzyme and 10 mM $MgCl_2$ caused the value of the diffusion constant for the enzyme to increase to the value initially measured for the free enzyme in the absence of magnesium. This was interpreted in terms of a contraction of the enzyme upon tRNA saturation.

In this paper, we reexamine the conclusions of Holler et al. (1981) by means of small-angle neutron scattering. Our data confirm the binding of two $tRNA^{Phe}$ molecules per *E. coli* phenylalanyl-tRNA synthetase molecule (Bartman et al., 1975). However, they fail to reveal any significant change of the radius of gyration of the enzyme upon binding $MgCl_2$ and $tRNA^{Phe}$. The measured radius of gyration and molecular weight of phenylalanyl-tRNA synthetase account for a globular protein with axial ratio of less or equal to 3.

Materials and Methods

Homogeneous phenylalanyl-tRNA synthetase was purified from *E. coli* strain EM20031 carrying the F32 episome (Fayat et al., 1974) or from the overproducing strain IBPC 1671 carrying plasmid DNA pB1 (Plumbridge et al., 1980). The purified enzyme catalyzed the isotopic $[^{32}P]PP_i$–ATP exchange at an initial rate of 80 ± 10 s^{-1} [25 °C; 20 mM imidazole hydrochloride, pH 7.6, 10 mM 2-mercaptoethanol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.05 mg/mL bovine serum albumin (Serva), 7 mM $MgCl_2$, 2 mM ATP, 2 mM $[^{32}P]PP_i$ (5–20 Ci/mol from the Radiochemical Centre, Amersham, U.K.), and 2 mM L-phenylalanine] (Blanquet et al., 1974) and $tRNA^{Phe}$ aminoacylation at an initial rate of 3.0 ± 0.5 s^{-1} [25 °C; 20 mM imidazole hydrochloride, pH 7.6, 10 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.05 mg/mL bovine serum albumin, 7 mM $MgCl_2$, 150 mM KCl, 2 mM ATP, 50 μM L-[^{14}C]phenylalanine (50 Ci/mol from the Commissariat à l'Energie Atomique, Saclay, France), and 100 μM unfractionated *E. coli* tRNA] (Lawrence et al., 1973).

Phenylalanine-specific tRNA was from Boehringer (Mannheim). It accepted 1100 ± 100 pmol of phenylalanine/A_{260nm} unit in 20 mM imidazole hydrochloride, 150 mM KCl, and 7 mM $MgCl_2$.

The extinction coefficient of phenylalanyl-tRNA synthetase was taken to be equal to 0.80 ± 0.10 cm^2 mg^{-1} at 280 nm (Fayat et al., 1974). That of $tRNA^{Phe}$ was taken to be equal to 24 cm^2 mg^{-1} at 260 nm (Guéron & Leroy, 1978).

Small-angle neutron scattering experiments were performed at the Institut Laue-Langevin on the D11 camera (Ibel, 1976). Samples were exposed to the neutron beam in a quartz cell

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0006-2960/83/0422-0281$01.50/0

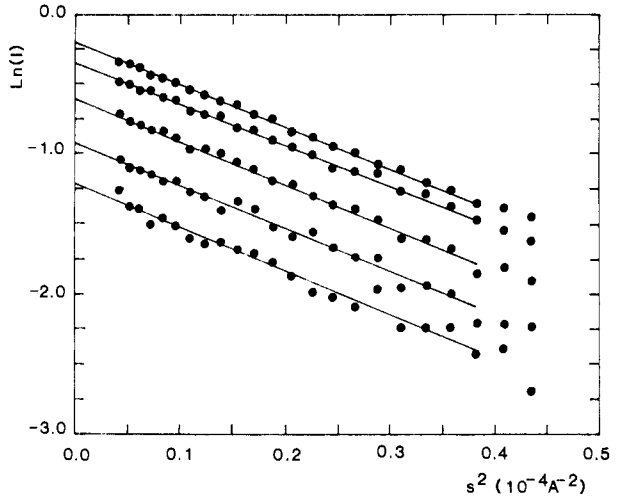

FIGURE 1: Neutron scattering curves of phenylalanyl-tRNA synthetase. Solutions are in 20 mM phosphate (K^+), pH 7.0, 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 150 mM KCl, containing enzyme at concentrations of 2.30, 3.13, 4.20, 5.44, and 6.30 mg/mL. $I(0)$ and R_G^2 values are obtained with associated standard errors by linear regression of the ln (I) values as a function of s^2 in the Guinier region.

according to Dessen et al. (1978, 1982). Scattering curves were observed with a wavelength of 10 Å in the range 0.0012 $Å^{-1} < s < 0.0075$ $Å^{-1}$ [s is defined as 2 sin (θ/λ)] with a sample to detector distance of 4.84 m and a collimation of 3.3 m. All experiments were performed at room temperature. The Guinier region (Guinier & Fournet, 1955) used in the analysis corresponded to 0.0020 $Å^{-1} < s < 0.0062$ $Å^{-1}$ so that $2\pi s R_G < 1.8$, with measured $R_G = 48$ Å. Measured points did not deviate from a straight line even above this value. A linear least-squares fit of the data in this range, for each scattering curve, yielded $I(0)$ and R_G^2 values with associated standard deviations. The variations of $I(0)$ and R_G as a function of added tRNA were analyzed according to Dessen et al. (1978, 1982).

Results

Structural Parameters of Phenylalanyl-tRNA Synthetase in Solution. Neutron scattering experiments were performed in ^1H_2O buffer at different enzyme concentrations between 2.3 and 6.3 mg/mL (Figure 1). The buffer was either 20 mM phosphate (K^+), pH 7.0, containing 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 150 mM KCl (Figure 1), or 20 mM imidazole hydrochloride, pH 7.6, containing 10 mM 2-mercaptoethanol and 0.1 mM EDTA (3.1 or 6.1 mg/mL enzyme concentration). Constant $I(0)/c$ and R_G values within ±4 and ±2% error, respectively, were measured up to an enzyme concentration of 6.3 mg/mL, whichever of the two buffers was used.

The molecular ratio of 227K ± 20K for phenylalanyl-tRNA synthetase deduced from an $I(0)/c$ mean absolute value of 0.128 ± 0.010 (Jacrot & Zaccaï, 1981) is in reasonable agreement with that obtained by Fayat et al. (1974) from solution light scattering and equilibrium sedimentation (240K ± 10K).

The mean R_G value is 48.3 ± 0.6 Å. On the assumption of 38% w/w hydration [calculated according to Kuntz (1971)] and by use of the enzyme amino acid composition (unpublished data), it corresponds to a prolate ellipsoid of revolution with an axial ratio of 3.0 (half-axes 32.2 and 97.7 Å). The axial ratio would be 2.6 for a molecular weight of 267K and 50% hydration as in Holler et al. (1981). Note that if two or four identical homogeneous ellipsoids had been used to model the

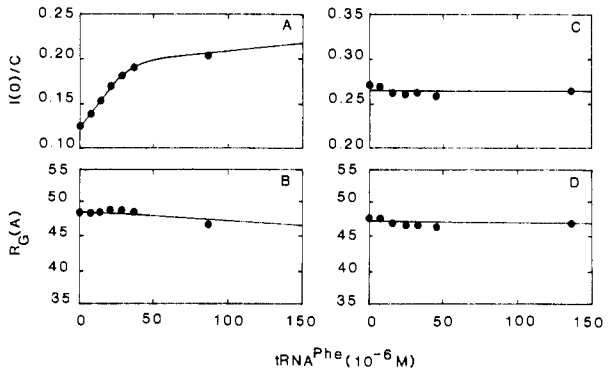

FIGURE 2: Neutron scattering titration of phenylalanyl-tRNA synthetase by $tRNA^{Phe}$. The enzyme is titrated by $tRNA^{Phe}$ in 20 mM imidazole hydrochloride, pH 7.6, 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 0.1 mM EDTA containing 0 (panels A and B) or 73% ^2H_2O (panels C and D). For each point of the titration, $I(0)/c$ (panels A and C) and R_G values (panels B and D) are measured with standard errors [1% and 2% for $I(0)/c$ and R_G values, respectively] and plotted as a function of tRNA concentration. Enzyme is 3.13 mg/mL (13.8×10^{-6} M) or 3.96 mg/mL (17.4×10^{-6} M), in 0 or 73% ^2H_2O, respectively. Lines correspond to $I(0)/c$ and R_G variations fitting with the experimental data. The fit was realized assuming n equivalent tRNA binding sites per enzyme molecule. The lines are calculated with $n = 2.4 \pm 0.2$, an equilibrium constant of 1×10^6 M^{-1}, and the parameters in Table I. R_G values used in the calculation are those given under Results.

$\alpha_2\beta_2$ tetrameric phenylalanyl-tRNA synthetase, the axial ratio would be smaller than the above value.

Identical molecular weight and R_G values were obtained independent of the origin of the enzyme, from strain EM20031 (haploid for the genes encoding phenylalanyl-tRNA synthetase) or from the polyploid overproducing strain IBPC 1671 carrying plasmid DNA pB1.

Finally, $MgCl_2$ was added to a solution of phenylalanyl-tRNA synthetase (5.7 mg/mL) in 20 mM imidazole hydrochloride, pH 7.6, 10 mM 2-mercaptoethanol, and 0.1 mM EDTA. $I(0)/c$ and R_G values did not change within ±2% error upon the addition of 10 or 56 mM $MgCl_2$.

Binding of $tRNA^{Phe}$ to Phenylalanyl-tRNA Synthetase. Titration of phenylalanyl-tRNA synthetase by $tRNA^{Phe}$ was performed in 20 mM imidazole hydrochloride, pH 7.6, 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 10 mM $MgCl_2$, containing 0 or 73% ^2H_2O. For each ^2H_2O concentration, $I(0)/c$ and R_G parameters were followed as a function of tRNA binding (Figure 2).

In ^1H_2O, $I(0)/c$ linearly increases by 60 ± 5% upon saturation with $tRNA^{Phe}$ (Figure 2A). This increase agrees within experimental error with the variation (63%) calculated on the assumption of two bound tRNA molecules per enzyme molecule (Table I). The binding is complete at a ratio of 2.4 ± 0.2 $tRNA^{Phe}$ molecules per enzyme molecule. Beyond this stoichiometery, $I(0)/c$ still increases, due only to the contribution of free $tRNA^{Phe}$. Owing to the high enzyme concentration used in the experiment, we do not distinguish between an independent or anticooperative process. Each tRNA binding step follows, however, an equilibrium constant <1 μM, in good agreement with earlier reports of Bartman et al. (1975) and Favre et al. (1979).

In 73% ^2H_2O, where the contribution of tRNA to the scattering is negligible, titration of phenylalanyl-tRNA synthetase by $tRNA^{Phe}$ (Figure 2C,D) fails to reveal any variation of $I(0)/c$ or R_G parameters outside experimental error. This implies the absence of marked rearrangement of the quaternary structure or of the conformation of phenylalanyl-tRNA synthetase upon binding $tRNA^{Phe}$.

Table I: Parameters[a]

	phenylalanyl-tRNA synthetase	$tRNA^{Phe}$
M_r	227 000	24 710
ν ($cm^3\ g^{-1}$)	0.74	0.53
V dry (Å^3)	279 000	21 740
V hydrated (Å^3) (38% w/w)	425 000	
Σb_{H_2O} (10^{-12} cm)	5068	796
Σb_{D_2O} (10^{-12} cm)	8867	1042
y	0.8	1.0
scattering amplitude (10^{-9} cm)		
in 0% ^2H_2O	6.6583	0.9197
in 73% ^2H_2O	−5.1152	+0.0124

[a] The dry volumes V are those which exclude the solvent, i.e., the volumes occupied by the atoms in the particles. They are calculated from $V = Mv/N$. The v value of tRNA (G. Zaccai, personal communication) corresponds to a matching point of 0.74 ^2H_2O. For the enzyme, a v value based on chemical composition is assumed (unpublished data). The total scattering lengths, Σb_{H_2O} and Σb_{D_2O}, of the enzyme and tRNA are calculated from their respective compositions (Barrell & Sanger, 1969; Jacrot, 1976). In the case of the enzyme, y is estimated from its v value and from the ^2H_2O concentration (41%) in which the scattering amplitude of the solvent matches that of the protein.

Note that the $I(0)/c$ value of the free enzyme in 73% ^2H_2O agrees within 3% error with the value calculated from Table I. An R_G of 47.7 ± 0.4 Å is measured, instead of 48.4 ± 0.7 Å in ^1H_2O. This is consistent with the hydrophilic groups on the surface of the protein (Stuhrmann, 1974).

Location of $tRNA^{Phe}$ on Phenylalanyl-tRNA Synthetase. From the absence of measurable difference in ^1H_2O between the R_G of the free enzyme and those of the 1/1 and 1/2 enzyme/tRNA complexes [R_G remains constant within 48.6 ± 0.6 Å (Figure 2B)], a distance of 43 ± 10 Å between the center of mass of the enzyme and that of each bound tRNA can be deduced by applying the parallel axis theorem. This calculation is made assuming (1) a symmetrical position of centers of mass of the tRNAs with respect to the center of the protein, (2) an absence of rearrangement of the protein moiety within the complex (as evident from the experiment in 73% ^2H_2O; Figure 2D), and (3) an R_G value of bound tRNA (22 Å) close to its solution value (G. Zaccaï, personal communication).

Discussion

The R_G value of phenylalanyl-tRNA synthetase in solution, as measured by neutron scattering, is 48.3 ± 0.6 Å. The axial ratio of a prolate ellipsoid of revolution equivalent in scattering (≤3) is in disagreement with that calculated by Holler et al. (1981), although measured under very similar buffer conditions. The axial ratio derived from the apparent translation diffusion constant, D, of the synthetase varied between 10 and 17 which would indicate a radius of gyration between 100 and 140 Å, when a molecular weight of the enzyme of 267K is considered. In addition, Holler et al. (1981) estimated from their diffusion constant value in standard buffer without additional salt a molecular weight of 291K which is significantly larger than that measured by Fayat et al. (1974), Hanke et al. (1974), and this study. These differences might reflect an underestimation of the D value used for molecular weight and friction factor ratio calculations. Holler et al. (1981) performed quasi-elastic light scattering with only one sampling time and one scattering angle. The use of only one exponential for the analysis of the correlation function is inadequate to exclude the possibility of several modes of diffusion. In particular, the presence of a small amount of aggregates, at low salt, would lead to an underestimation of the enzyme D value. In addition, electrostatic effects should also be taken in consideration in any quasi-elastic light scattering study on biological macromolecules which is conducted around 0.01 M ionic strength or below (Raj & Flygare, 1974; Doherty & Benedek, 1974; Fulmer et al., 1981; Tivant et al., 1982). These studies have indicated that the apparent translational diffusion constant of the ionic macromolecules increases as the ionic strength of the solution is decreased. This effect is accompanied by a decrease in scattering intensity toward lower ionic strength and attributed to electrostatic coupling between rapidly diffusing small ions and the macromolecule.

The neutron scattering approach indicates that addition of $MgCl_2$ and of $tRNA^{Phe}$ has no measurable effect on the R_G of the enzyme, while, in turn, these ligands markedly affected the diffusion constant. Binding of a polyanion such as tRNA may lead to changes in the apparent translational diffusion constant and in the intensity of the scattered light which do not reflect changes in macromolecular size and/or shape but result from a modification of electrostatic coupling between diffusion modes of the small ions and the macromolecular complex. It cannot be excluded, however, that small conformational changes occur which are not seen in the R_G value. For instance, the change of a prolate ellipsoid into an oblate one affects the translational frictional coefficient ratio, but not the R_G value (Sheraga, 1961).

Finally, *E. coli* phenylalanyl-tRNA synthetase is an $\alpha_2\beta_2$ tetrameric enzyme (Fayat et al., 1974). It can be modeled as a cluster of four identical subunits with the same hydrodynamic properties. Two limiting shapes can be distinguished: (1) linear arrangement of the four subunits; (2) tetrahedron arrangement. The frictional coefficient of the linear tetramer is only 20% larger than that of the tetrahedron (Teller et al., 1979; Cantor & Schimmel, 1980). On the other hand, the R_G of the linear tetramer is 63% larger than that of the tetrahedron. Thus, direct R_G measurement by analyzing the angular dependence of the scattered intensity (Guinier plot) appears to be the more sensitive approach to the determination of the overall shape of an oligomeric protein in solution.

Acknowledgments

We gratefully acknowledge Drs. B. Jacrot and G. Weisbuch for helpful discussions and Drs. G. Zaccaï and M. Drifford for communicating manuscripts prior to publication.

Registry No. Phenylalanyl-tRNA synthetase, 9055-66-7; neutron, 12586-31-1.

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Binding of Daunomycin to Calf Thymus Nucleosomes†

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ABSTRACT: We report equilibrium, hydrodynamic, and electric dichroism studies of the complex of daunomycin with H1-depleted 175 base pair nucleosomes, along with some comparative data for ethidium. In contrast to ethidium, daunomycin binding to nucleosomes is strongly reduced relative to the affinity for free DNA. The salt concentration dependence of the binding constant indicates that approximately one Na^+ ion is released from both nucleosomes and free DNA upon daunomycin binding. The early melting transition of nucleosomes is preferentially stabilized by low levels of both drugs, but more markedly by ethidium. Ethidium also stabilizes the second nucleosome melting transition, but daunomycin barely does so. Dichroism and rotational relaxation time measurements indicate that daunomycin unfolds nucleosomes in a manner analogous to the influence of ethidium, although about twice as much daunomycin as ethidium is required to complete the unfolding process. The data support an unfolded structure in which the nucleosome elongates along the DNA superhelical axis. Levels of daunomycin greater than about 0.15 per DNA base pair promote nucleosome aggregation. To relate our results to the activity of daunomycin as an antitumor agent, we propose that the druug, because of its special intercalation geometry, strongly prefers free DNA regions over the bent helices found in nucleosomes and chromatin. The result of this preference should be an increased local concentration of the drug in the genetically active regions of nuclear DNA in which nucleosomal structure is less prevalent. Presumably the abundance of such regions in tumor cells makes them especially sensitive to daunomycin.

The anthracycline antibiotic daunomycin is widely used in the treatment of human cancers. It is proposed that the drug acts by direct interaction with nuclear DNA and subsequent inhibition of DNA replication and RNA transcription [for reviews, see Crooke & Reich (1980), Arcamone (1978), and Neidle (1978)].

Numerous studies of the interaction of daunomycin with DNA have appeared (Zunnio et al., 1972, 1980; Gabbay et al., 1976; Plumbridge & Brown, 1977; Barthalemy-Clavey et al., 1973; Huang & Phillips, 1977; Molinier-Jumel et al., 1978; Schütz et al., 1979). We have reported the details of our research on the self-association of daunomycin (Chaires et al., 1982a), equilibrium aspects of the daunomycin–DNA interaction (Chaires et al., 1982b), and the geometry of the daunomycin–DNA complex (Fritzsche et al., 1982).

In the cell, however, it is not naked DNA that serves as a binding site for daunomycin, but rather chromatin, consisting of DNA complexed with histones and other nuclear proteins. How the presence of proteins on the DNA might affect the binding of daunomycin is a question of importance in efforts to understand the action of the drug.

To explore this question, we have examined the interaction of daunomycin with H1-depleted nucleosomes containing lengths of DNA of 146 and 175 base pairs (bp). We find that the presence of core histones drastically reduces the affinity of daunomycin for DNA. Hydrodynamic studies show that

† From the Department of Chemistry, Yale University, New Haven, Connecticut 06511. *Received May 24, 1982; revised manuscript received August 23, 1982.* Supported by Grant CA 15583 from the National Cancer Institute. This paper is dedicated to Professor Gerson Kegeles on the occasion of his retirement from the University of Connecticut.

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0006-2960/83/0422-0284$01.50/0