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Sizes of Two Amino Acyl-tRNA Synthetase Complexes from E. coli with Their Cognate tRNAs

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Summary: The complexes of valyl-tRNA synthetase with $tRNA_I^{Val}$ and arginyl-tRNA synthetase with $tRNA_{II}^{Arg}$ from E. coli were studied by light scattering measurements and analytical ultracentrifugation of concentrations as low as 40 µg/ml. The molecular weights determined from these studies were 260,000 ± 2,000 for the valyl-tRNA synthetase·tRNA complex, and 310,000 ± 1,500 for the arginyl-tRNA synthetase·tRNA complex at pH 7.1. The stoichiometry for the complexes are apparently 2:1 for valyl-tRNA synthetase and tRNA and 4:1 in the case of the arginyl-tRNA synthetase and tRNA. From the angular dependence of the scattered intensity a radius of gyration of 54.5 Å for the complex between valyl-tRNA synthetase and tRNA was found, whereas for the other complex a value of 59.1 Å was found.

Tight complexes of the amino acyl-tRNA synthetases with their cognate tRNAs in the absence of amino acid and ATP are a well known property although this complex formation is not quite specific since associations between these enzymes and non-cognate tRNAs also have been observed (1,2). The formation of such amino-acyl tRNA synthetase·tRNA complexes has been detected by a variety of physical and chemical techniques (2).

We report here the molecular weights of two purified amino acyl-tRNA synthetase·tRNA complexes by means of light scattering and sedimentation equilibrium experiments, e.g., the size of valyl-tRNA synthetase·$tRNA_I^{Val}$ complex (VRS·tRNA) and of arginyl-tRNA synthetase·$tRNA_I^{Arg}$ (ARS·tRNA), including their radii of gyration from the dependency of the scattered light extrapolated to zero concentration.

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Materials and Methods

VRS was purified as described by Paradies (3,4). Polyacrylamide SDS gel electrophoresis according to Weber and Osborn (5) showed one band, indicating no proteolytic fragmentation of the enzyme. ARS was purified similar to VRS, but eluted from the BioGel 0.5 m column at lower potassium phosphate, 0.015 M at pH 7.5 (6). $tRNA^{Val}$ and $tRNA^{Arg}$ were purchased from Boehringer (Mannheim), further purified and subjected to deacylation prior to complex reaction in phosphate buffer as previously described (7,8).

Preparation of the enzyme·tRNA complex. Stoichiometry amounts of VRS and ARS were incubated at 30°C for 30 min in 0.01 M K_2HPO_4, pH 7.5, with the cognate tRNAs which have been deacylated prior to incubation as described by Paradies (8). Protein and tRNA concentration were determined from their absorption units at 280 nm and 260 nm, respectively (3,9). The complex VRS·tRNA was purified on a BioGel A 1.5 m column (1.2 x 50 cm) at pH 7.1 in 0.01 M potassium phosphate buffer containing 0.1 M KCl and 6 mM β-mercaptoethanol. This complex elutes as a single peak close to position of 280,000 of the calibrated column with spherical protein of known molecular weight and similar partial specific volumes. The ARS·tRNA complex was eluted at pH 7.5, in 0.01 M K_2HPO_4, containing 6 mM β-mercaptoethanol and 0.1 M KCl. Applying the condition of active enzyme gel chromatography (10) the obtained values of the partition cross-section determined for these two complexes are in good agreement with values reported for a 280,000 molecular weight species in the case of VRS·tRNA and 1.15 x 10^6 for ARS·tRNA.

Light scattering measurements. Measurements were made in a Wippler-Scheibling light scattering photometer (Fica, Paris) as described by Paradies et al. (11). The specific refractive index increment $(\partial n/\partial c)_n$ for both complexes in 0.01 M K_2HPO_4, pH 7.1 and pH 7.5, respectively, were determined by differential refractometry (Brice, Phoenix) at 20°C (thermostated) (11). The value of $(\partial n/\partial c)_{\mu = const}$ used at 20°C at 5465 Å was 0.175 $ml \cdot g^{-1}$ for VRS·tRNA and 0.179 ml/g for ARS·tRNA.

Analytical ultracentrifugation was carried out in a Spinco Model E Analytical Centrifuge (Beckman) at 4°C, using the photoelectric scanner at 275 mm. Molecular weights were obtained from sedimentation equilibrium analysis at two rotor speeds of 5,200 and 9,500 rpm from a plot of lnc vs $r^2/2$, and $1/M_{W,app}$ vs c (in mg/ml), and from active enzyme centrifugation (11).

Results

Active enzyme gel chromatography on BioGel A 1.5 m of the complexes was carried out at concentrations as high as 5 mg/ml and as low as 15 µg/ml (100 µl samples) according to Jones et al. (10). The average of the partition coefficients which were calculated by the size of the partition cross-section from the

centroid yielded a value of 275,000 ± 5,000 for VRS·tRNA and (1.1 ± 0.16) x 10^6 for ARS·tRNA on a BioGel A 5 m column. The biological activity of the supposed complex peaks in the acylation assay (4) for both enzyme complexes coincided with the protein peak.

Values of Hc/ΔR were plotted by the double extrapolation method according to Zimm (13) for both enzyme complexes (Fig. 1). The values recorded between θ (20°) and θ (45°) have been included in the extrapolations to infinite dilution and zero scattering angle. The reduced zero angle plots which are shown in Fig. 1 for both complexes yielded an apparent average molecular weight of 260,000 ± 2,000 for VRS·tRNA and 310,000 ± 1,500 for ARS·tRNA, respectively. In contrast to VRS·tRNA the corre-

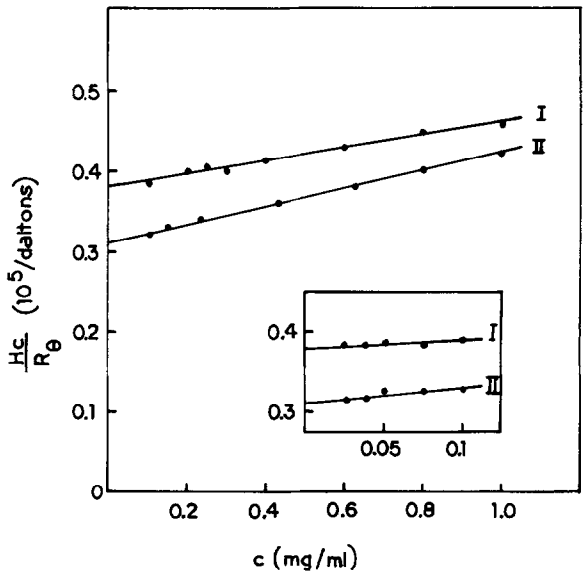

Fig. 1. Zero angle extrapolation of VRS_2·tRNA (I) and ARS_4·tRNA (II) complexes at 546 nm (λ_o). $Hc/R_\theta = M_W^{-1} + 2A_2c + \ldots$ with $H = \left(\frac{\partial n}{\partial c}\right)_\mu^2 2\pi^2 n_o^2/\lambda_o^4 \cdot N_A$ with N_A = Avogadro's number, n_o the solvent refractive index and $(\partial n/\partial c)$ the refractive index increment at constant chemical potential. A_2 is the second virial coefficient and c the concentration in mg/ml. Insert: zero angle extrapolation at very low complex concentrations.

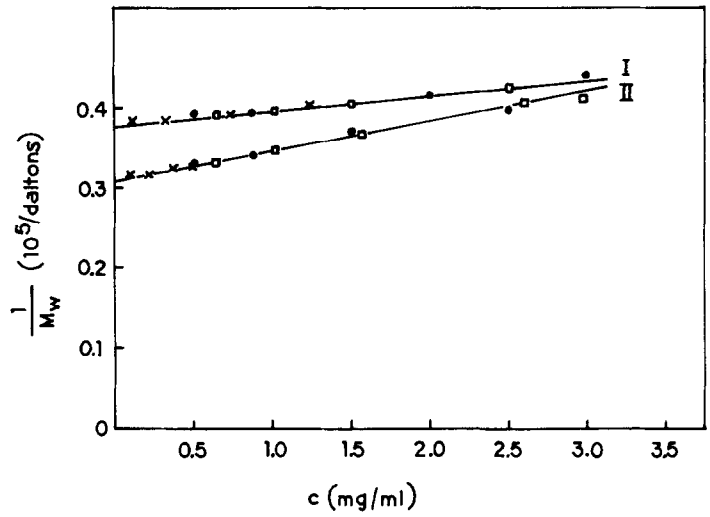

Fig. 2. Apparent reciprocal weight average molecular weights for the two complexes (I) and (II) at two rotor speeds. I, ● 8,000 rpm, □ 10,000 rpm; II, ● 7,000 rpm, □ 9,000 rpm; all experiments with 100 µl loading volume and at 20° C. x—x are zero angle apparent reciprocal weight average molecular weights ($Hc/R_{\theta=0}$) from light scattering measurements.

sponding ARS·tRNA complex has a positive slope, showing non-ideality; from the slope a second virial coefficient of A_2 = 5.65 x 10^{-4} mole ml/g^2 (Fig. 1) is calculated, whereas for the corresponding VRS·tRNA complex no significant concentration dependence is observed. Together with the data obtained by analytical ultracentrifugation (Fig. 2) the apparent weight average molecular weights of the two complexes correspond to 260,000 for VRS·tRNA and 315,000 for ARS·tRNA. The angular dependences of both complexes are shown in Fig. 3 extrapolated to zero concentration from which the apparent z-average square radius of gyration is calculated, which was found to be 2970.3 $Å^2$ for VRS·tRNA and 3492.8 $Å^2$ for the ARS·tRNA complex. Since we are not dealing with a polydisperse system and measurements were conducted at constant chemical equilibrium and h = 0, the number and z-weight average molecular weight equals the weight

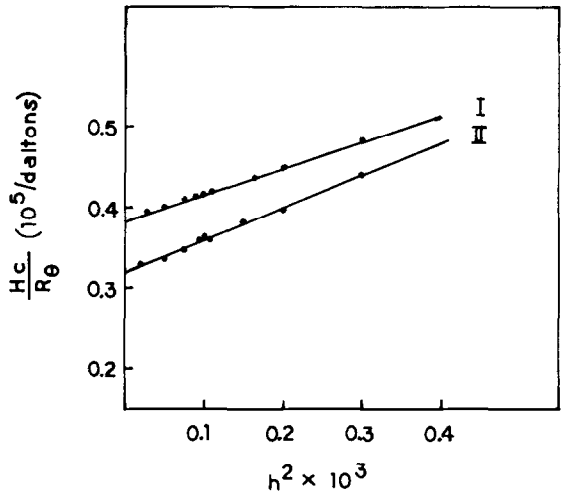

Fig. 3. Angular dependence extrapolated to zero concentration at 546 nm (λ_0), 20°C (I) and (II).

average molecular weight and the mean square radius of gyration is independent from the shape of the macromolecule at small scattering angles.

Dissociation of the complexes by addition of guanidinium chloride (4.0 M) at pH 7.1 in phosphate buffer and gel permeation chromatography on BioGel 5 m resulted in two peaks: one in the void volume corresponding to the proteins of molecular weights of 110,000 and 71,000, obtained by sedimentation equilibrium measurements, and one tRNA peak corresponding to a molecular weight of 25,000 according to the procedure of Fish et al. (14). Considering the molecular weights, the radii of gyration and the values of the apparent weight average molecular weight from sedimentation equilibrium experiments, the stoichiometry of the valyl-tRNA synthetase complex from E. coli corresponds to two molecules of enzyme and one of tRNA, $VRS_2 \cdot tRNA$, and that of the arginyl-tRNA synthetase of $ARS_4 \cdot tRNA$ which has to be rather asymmetric considering the elution profile where the complex elutes at a position of 1.1×10^6 and the apparent radius of gyration.

Discussion

The stability of the studied enzyme-tRNA complexes is pH dependent, e.g., the dissociation of both complexes occurs at higher pH (7.7). Almost all of the complexes are dissociated at pH 8.0, where the enzyme and tRNA can be separated on a BioGel A 1.5 m column. The complexes are fairly stable at 20°C since they do not dissociate during the light scattering measurements and are stable during gel chromatography although the enzymes have been purified at 4°C. Upon dilution (20°C) both complexes appear to be homogeneous and do not dissociate into particles of smaller molecular weights, e.g., to a VRS·tRNA complex or ARS_3·tRNA, ARS_2·tRNA or even enzyme and tRNA. Measurements of very dilute solutions of enzyme complex in the range of 15 µg/ml to 100 µg/ml (see insert Fig. 1) still show an apparent weight molecular weight of 250,000 for VRS_2·tRNA and 310,000 for ARS_4·tRNA, which is further substantiated by active enzyme gel chromatography (10). The results presented here are consistent with the formation of an enzyme-tRNA complex containing two ligase molecules and one tRNA molecule in the case for valyl-tRNA synthetase and four ligase molecules and one tRNA in the case for the arginyl-tRNA synthetase. By comparing the different radii of gyration of these complexes the shape of the ARS_4·tRNA complex has to be asymmetric, since for a spherical macromolecule of molecular weight 310,000, $v_2 = 0.715$ ml·g^{-1} (7) and a degree of hydration of 0.35 g H_2O/g complex would result in a volume of $v = 5.5 \times 10^5$ Å^3 and a radius of gyration of the excluded volume of $R_g = 39.2$ Å. The same is true for a spherical complexed particle, which is consistent with the observed dimerization process of valyl-tRNA synthetase from E. coli (15).

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