Phenylalanyl-tRNA Synthetase from Baker'Yeast: Structural Organization of the Enzyme and Its Complex with $tRNA^{Phe}$ as Determined by X-Ray Small-Angle Scattering

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The quaternary structure of the phenylalanyl-tRNA synthetase and its complex with $tRNA^{Phe}$ was studied in dilute solutions by small angle X-ray scattering. For the free synthetase the radius of gyration was determined as 5.5 nm, the volume 523 nm^3, the maximum diameter 17.5 nm and the molecular weight as 260 000 using an isopotential specific volume of 0.735. The overall shape could be best approximated by a flat cylinder with dimensions 18.2 nm×11.5 nm×4 nm; the loose structure was approximated by building up the cylinder by spheres (diameter 4.2 nm).

The corresponding parameters of the enzyme tRNA complex were the following: radius of gyration 5.9 nm, volume 543 nm^3, maximum diameter 21 nm and molecular weight 290 000. These parameters suggest an 1:1 complex, whereby it must be assumed that the tRNA molecule is attached in the extension of the longer axis. From the difference in the distance distribution functions of the free enzyme and the complex it is evident that we have to assume a change of conformation (contraction) of the enzyme upon the binding of the specific tRNA.

Introduction

Aminoacyl-tRNA synthetases are a group of enzymes catalysing the aminoacylation of specific tRNA, the first step of protein biosynthesis [1]. Despite their functional identity they vary widely with respect to their protein nature. Single chain enzymes, for example valine-tRNA synthetase with molecular weight 122 000, are found besides double chain enzymes of the a_2 type (for example tyrosyl-tRNA synthetase from E. coli with a molecular weight of 47 500 for each chain) and four chain enzymes of the a_2b_2 type (for example Phenylalanyl-tRNA synthetase with molecular weight 260 000, 73 000 for the heavy and 63 000 for the light chain) [1].

Little is known about the structural organisation of these enzymes. Crystallisation studies with several aminoacyl-tRNA synthetases were undertaken [1]. The most advanced investigations were performed with a catalytically active fragment of methinoyl-tRNA synthetase from *E. coli* [2] and the tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* [3] for which low resolution X-ray structures have been determined. As an alternative lysyl-tRNA synthetase from *Bacillus stearothermophilus* has been subjected to an X-ray scattering investigation [4].

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We wish to describe here an X-ray small-angle scattering investigation of phenylalanyl-tRNA synthetase from baker's yeast. In addition to the free enzyme we analysed an 1 : 1 (mol/mol) mixture of enzyme and $tRNA^{Phe}$ for the following reasons. On affinity elution [5] 1 mol of $tRNA^{Phe}$ is able to elute 1 mol of enzyme pointing to half of the site reactivity. This is in contrast to the fact, that during equilibrium studies of binding of enzyme to tRNA two binding sites with equal K_{diss} were determined [6, 7]. It was our hope, to get more insight regarding these conflicting results by investigation of an 1 : 1 mixture of tRNA and enzyme.

Materials and Methods

Phenylalanyl-tRNA synthetase (E.C. 6.1.1.20) and $tRNA^{Phe}$-C-C-A were prepared as described [8], respectively [9]. All other salts and reagents were of analytical grade commercially available.

Preparation of enzyme

Phenylalanyl-tRNA synthetase was stored in a stock solution consisting of 50% glycerol and 50% 0.03 M potassium phosphate buffer pH 7.2 at a concentration of about 10 mg/ml. An appropriate aliquot of this solution was diluted 1 : 4 (v/v) with 0.03 M potassium phosphate buffer pH 7.2. The enzyme was adsorbed into an 1 ml DE 52 cellulose column (Whatman). Excess glycerol was washed of

with 0.03 M potassium phosphate buffer. The enzyme was released from the column with the phosphate buffer containing 0.2 M KCl and 0.01 M $MgSO_4$. 100 μl fractions were collected and the content of enzyme was determined spectrophotometrically. 1 A_{260} unit is equivalent to 0.89 mg enzyme. Molecular weight is 260 000 [10].

tRNA was dissolved to a concentration of 400 A_{260}-units per ml in the 0.03 M potassium phosphate buffer containing 0.2 M KCl and 0.01 M $MgSO_4$. It was added from this stock solution in an equivalent amount (mol/mol) to the enzyme solution. 1 A_{260} unit tRNA is equivalent to 1.7 nmol [10].

Small-angle X-ray scattering

The measurements were carried out using a highly stabilized X-ray generator (Philips PW 1140) with a copper target tube (50 kV, 30 mA) and a Kratky camera with slit collimation system [11].

The protein solutions were placed in Mark capillaries (diameter about 0.1 cm) at 4 °C.

The scattered intensities were measured at 80 different angles in the range between 0.0021 and 0.103 radians using an entrance slit of 120 μm. An electronically programmable stepscanning device [12] allowed automatic operation. A proportional counter with an X-ray analysis channel control was used for recording. A pulse height discriminator was focussed on the CuKα line. Concentration series were measured, and the values extrapolated to zero concentration. Each scattering curve was recorded several times (when 10^5 pulses per point were counted the relative statistical error was about 0.3%). Alteration in the scattering behaviour (apparently due to denaturation and aggregation) occured only at high concentrations (greater than 40 mg/ml). Evaluation of the scattering data was performed by computer programs of Zipper [13] and Glatter [14]. The enzyme and tRNA concentrations were determined by measuring the absorbance at 260 nm [10].

Isopotential specific volume

The isopotential specific volume v_1' [15] was determined by the oscillator method [16] using a digital densitometer device DMA 02/C at 4 °C.

Results and Discussion

Free enzyme

Concentration series of four independent prepared samples were measured. The Guinier plots of the innermost portions of the immediately obtained (smeared, unsmoothed) scattering curves are shown in Fig. 1. The apparent radii of gyration determined from the Guinier plots of the curves of the various concentrated solutions are summarized in Table I for the four samples and plotted versus the concentration c in Fig. 2; the agreement is satisfying.

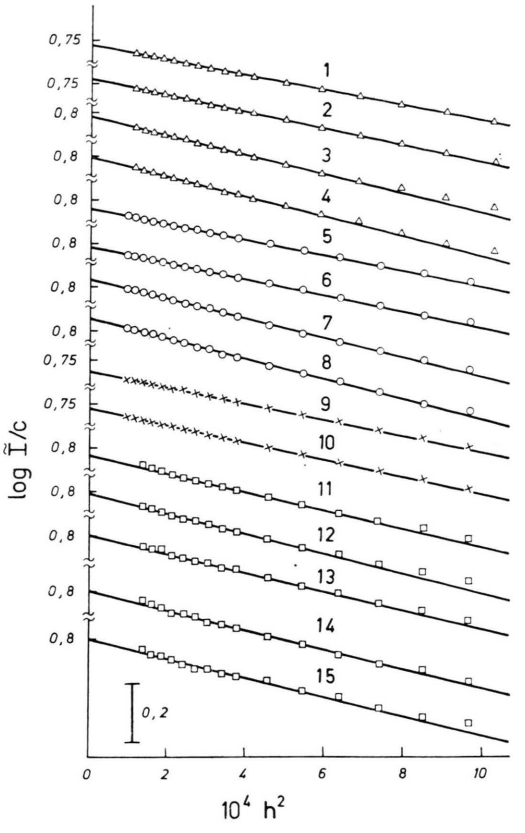

Fig. 1. Innermost portions of the scattering curves of four independent prepared samples of synthetase in Guinier plot. From each sample a concentration series was measured; the concentrations of the enzyme are summarized in Table I. The numbers in the Table correspond to the numbers in the figure. The curves obtained for the same sample are marked with the same symbol. $\tilde{I}$, smeared scattered intensity; c, concentration of the synthetase in solution; $h = (4\pi \sin\theta)/\lambda$; λ = wavelength in Å.

The final desmeared, monochromatized and to zero concentration extrapolated scattering curve of the free enzyme is shown in Fig. 3. The following molecular parameters (summarized in Table II) could be derived from it.

Radius of gyration

The true value of the radius of gyration R was calculated from the Guinier plot ($\log I/c$ versus

Table I. Smeared $\tilde{R}$ and desmeared R-values of the radius of gyration calculated for the synthetase from the slopes of the Guinier plots in Fig. 1. The numbers in the figure correspond to the numbers in the table; c is the concentration of the free synthetase.

No.	c[mg/ml]	$\tilde{R}$[nm]	R[nm]
1	30.4	4.22	4.28
2	22.4	4.41	4.59
3	11.6	4.72	5.03
4	6.4	4.84	5.26
5	30.4	4.24	4.38
6	22.8	4.43	4.61
7	11.6	4.73	5.09
8	6.4	4.85	5.21
9	27.5	4.28	4.37
10	19.4	4.46	4.65
11	15.9	4.54	4.83
12	13.3	4.62	4.93
13	11.9	4.66	4.91
14	10.6	4.72	5.06
15	7.9	4.78	5.17
		$\tilde{R}_{c\to 0}=5.0$ nm	$R_{c\to 0}=5.5$ nm

h^2), whereby I is the scattered intensity obtained after desmearing, monochromatization and extrapolation to zero concentration. All four samples of the free enzyme yielded a value of $R = 5.5$ nm.

The radius of gyration may also be calculated from the distance distribution function $p(r)$, which is the correlation function $\gamma(r)$ [17] multiplied by the square of the distance r:

$$R^2 = \frac{\int_0^\infty p(r)\, r^2\, dr}{\int_0^\infty p(r)\, dr}.$$

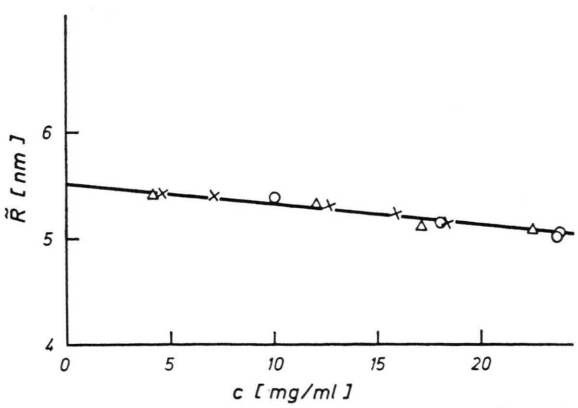

Fig. 2. Values of the apparent radius of gyration $\tilde{R}$ plotted versus the concentration c of the synthetase. The $\tilde{R}$-values were calculated from the slopes of the scattering curves in Fig. 1 and are listed in Table I.

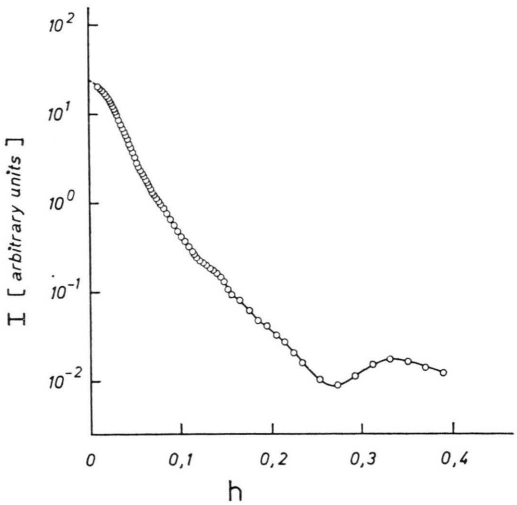

Fig. 3. Scattering curve of the free synthetase. I, scattered intensity; $h = (4\,\pi \sin\theta)/\lambda$; λ, wavelength used.

The $p(r)$ function was calculated from the smeared scattering curves by means of a computer program using the indirect Fourier transformation [18].

To determine the R-value from the $p(r)$ function has the advantage, that not only the innermost part of the scattering curve is used, but the whole scattering curve.

The values for the radius of gyration obtained from the $p(r)$ function by this method agree well with those obtained from the Guinier plot as listed in Table II.

Table II. Molecular parameters of the free synthetase and the synthetase: tRNA complex: radius of gyration R, molecular weight M_r calculated for two different v_1'-values, maximum diameter d_{max}, volume V and degree of hydration q.

Molecular parameter	Derived from	Dimension	Free synthetase	Complex
R	Guinier plot	nm	5.50 ± 0.02	5.92 ± 0.02
R	$p(r)$	nm	5.58 ± 0.08	5.95 ± 0.1
M_r ($v_1'=0.735$)	I_0, P_0	dalton	260 000	290 000
M_r ($v_1'=0.740$)	I_0, P_0	dalton	270 000	305 000
d_{max}	$p(r)$	nm	17.5 ± 0.5	21 ± 1
V	I_0, Q	nm^3	523 ± 5	543 ± 5
q ($v_1'=0.735$)	M, V	g Protein per g H_2O	0.44	0.41
q ($v_1'=0.740$)	M, V	g Protein per g H_2O	0.38	0.35

Maximum diameter

From the $p(r)$ function a further parameter, the maximum diameter of the particle can be obtained. $p(r)$ becomes zero at values of r equal or greater than the maximum diameter d_{max}. Thus determined d_{max} amount to 17.5 nm for the free enzyme (see Fig. 4).

Molecular weight

It was determined from the scattered intensity at zero angle, the contrast in electron density Δz between the solute protein and the solvent and the absolute intensity. The latter was found by using a calibrated Lupolen® sample [19, 20]. To obtain Δz with sufficient accuracy, the isopotential specific volume v_1' must be known with highest accuracy. Unfortunately the amounts of sample available were too small to determine the isopotential specific volume of the enzyme with high precision. The v_1'-values determined from the measured densities of solvent and solution lay between 0.735 and 0.740. Thus the molecular weight and the degree of hydration were calculated using both limiting values (Table II).

Volume and degree of hydration

The volume can be determined from the intensity at zero angle I_0 and the invariant Q given by the integral

$$Q = \int_0^{\infty} I(2\theta)\,(2\theta)^2\,d(2\theta) = \int_0^{(2\theta)^+} I(2\theta)\,(2\theta)^2\,d(2\theta) + \frac{k_1}{(2\theta)^+}.$$

Since the scattered intensity cannot be measured up to the angle ∞ the integration was performed up to a relatively large scattering angle $(2\theta)^+ = 0.059$ radians numerically by the Simpson rule. The tail end of the curve was integrated analytically after determining the tail-end constant k_1 from the $k_1(2\theta)^{-4}$ course found at large angles.

For the volume a value of 523 nm^3 was determined. This value corresponds to the hydrated volume of the particle. An unhydrated volume could be calculated from the molecular-weight and isopotential specific volume. A comparison of the values obtained for the both volumes yielded a degree of hydration of 0.44 (resp. 0.38) gram H_2O per gram protein (Table II).

Overall shape

Model calculation were performed by means of computer programs, which calculated the theoretical curves of simple triaxial bodies, bodies composed of some ellipsoid or cylinders [21] and models composed of a large number of spherical subunits [22]. The data of the models such as radius of gyration, volume, maximum diameter etc. were the same as those determined experimentally. To prove wether a model is "equivalent in scattering" or not its scattering curve (reciprocal space) and its distance distribution function $p(r)$ (real space) are compared with the experimental ones. Models which fit the experimental curves well can be discussed as in a high degree reliable. With certainty only models which do not fit the experimental curves can be excluded.

The comparison with simple triaxial bodies showed that the free synthetase could be described to a first approximation by a flat cylinder. A better fit was obtained by a somewhat more loose structure (model in Fig. 4). The spheres in the model are not intended to represent real subunits; they are only used mimic a loose structure. In Fig. 4 the experimental distance distribution function in compared with the $p(r)$ function calculated for the model shown in the figure.

The molecular parameters derived here (Table II) agree very well with the data given in the literature [8, 23], which were determined by completely

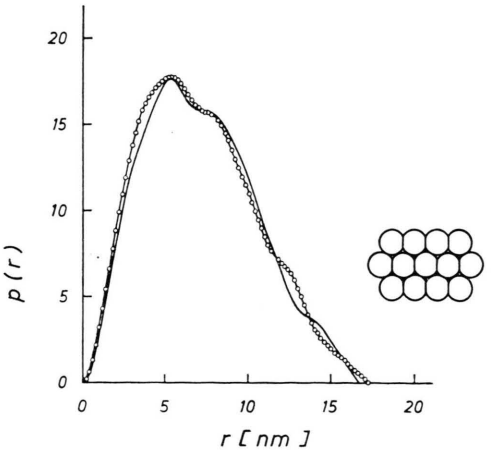

Fig. 4. Distance distribution functions $p(r)$ of the free synthetase (○○○) and the model (———) shown in the figure. The model consists of 13 spherical subunits with a diameter of 4.25 nm. The length of the model is 18.6 nm, the breadth 11.6 nm. r, distance between two volume elements in the particle.

independent methods. On this basis we feel safe, that also the molecular model (Fig. 4) is close to the enzymes shape in solution. A symmetrical model as given in Fig. 4 can account for the mechanistic behaviour exhibited by the enzyme *e. g.* two binding sites [5, 6] and half of the site reactivity under certain conditions [10].

Enzyme: tRNA complex

Concentration series of three independent samples (ratio enzyme: tRNA always 1 : 1 mol/mol) were measured. The Guinier plots of the innermost portions of the immediately obtained smeared scattering curves, which show best the degree of accuracy of the measurements, are presented in Fig. 5.

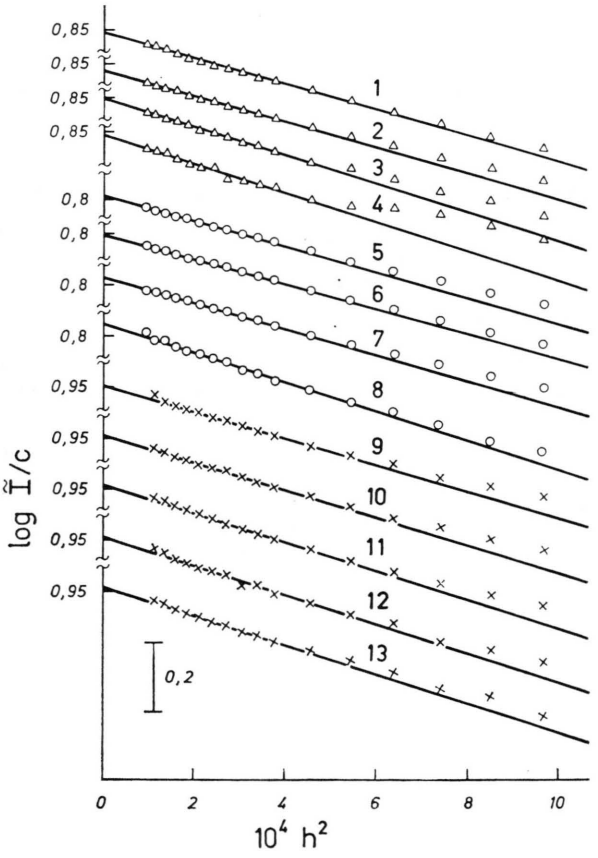

Fig. 5. Innermost portions of the scattering curves of three independent prepared samples of the complex in Guinier plot. From each sample a concentration series was measured. The three series are marked with different symbols. The concentrations c and the apparent radii of gyration $\tilde{R}$ calculated from the slopes of the scattering curves are summarized in Table III. $\tilde{I}$, slit smeared scattered intensity; c, concentration of the complex $h = (4\,\pi \sin\theta)/\lambda$.

Table III. Smeared $\tilde{R}$ and desmeared R-values of the radius of gyration calculated for the synthetase: tRNA complex from the slopes of the Guinier plots in Fig. 5. The numbers in the figure correspond to the numbers in the table; c is the concentration of the complex.

No.	c[mg/ml]	$\tilde{R}$[nm]	R[nm]
1	22.5	5.08	5.32
2	17.1	5.12	5.48
3	12.0	5.32	5.66
4	4.1	5.41	5.78
5	23.8	5.06	5.22
6	23.7	5.02	5.26
7	18.0	5.15	5.43
8	10.0	5.39	5.63
9	18.3	5.15	5.52
10	15.9	5.24	5.58
11	12.6	5.32	5.67
12	7.1	5.41	5.75
13	4.6	5.43	5.82
		$\tilde{R}_{c\to 0} = 5.52$	$R_{c\to 0} = 5.93$

The values of the apparent radii of gyration calculated from the slope of the curves are summarized in Table III and plotted versus the concentration of the complex in Fig. 6. The scattering curve of the complex is shown in Fig. 7; it has a shoulder at the same position as the free enzyme and its course is similar. Only at large angles their are differences. The $p(r)$ function is shown in Fig. 8. It is also similar to that of the free enzyme at small distances r, but differs clearly at larger r-values.

Molecular parameters

The molecular parameters of the complex were determined in the same way as described for the

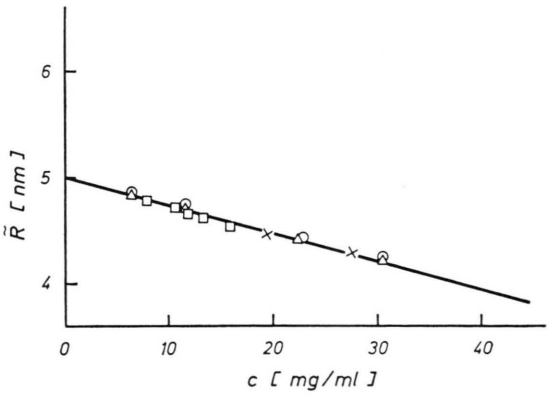

Fig. 6. Apparent radii of gyration $\tilde{R}$ calculated from the Guinier plots in Fig. 5 and plotted versus the concentration c of the complex. The concentrations are summarized in Table III.

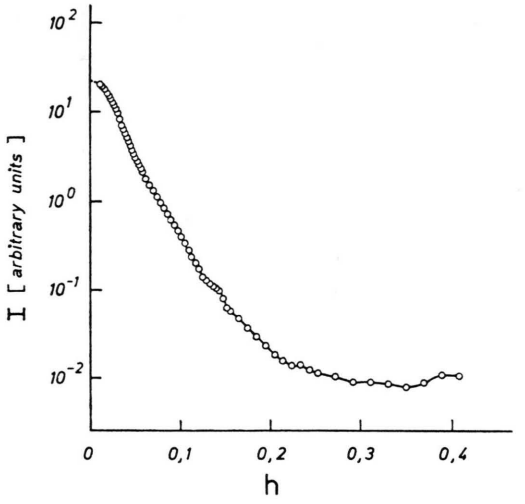

Fig. 7. Scattering curve of the complex. I, scattered intensity; $h = (4\pi \sin\theta)/\lambda$.

free synthetase and are summarized also in Table II. A comparison between the values of the free enzyme and the complex yields the following differences. The difference of about 30 000 molecular weight units between free synthetase and complex supports that an 1:1 complex was formed under the experimental conditions. While the absolute value of the molecular weight for the reasons discussed above (unaccurate value of the isopotential specific volume) could not be determined precisely the difference between the molecular weights for a certain v_1'-value could be obtained much more accurately.

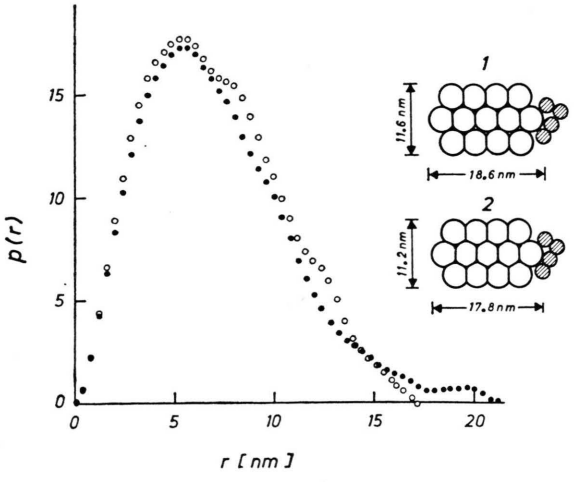

Fig. 8. Comparison of the experimental $p(r)$ functions of the free synthetase (○ ○) and the complex (● ●). The two models for the complex are discussed in the text.

The radius of gyration increased from 5.5 nm for the free enzyme to 5.9 nm for the complex. The increase in volume is relatively small – from 523 nm^3 to 543 nm^3 – while the increase in the maximum diameter is significant – from 17.5 nm to about 21 nm as seen in Fig. 8. The degree of hydration was found to be somewhat lower, 0.41 (resp. 0.35) gram H_2O per gram protein.

The X-ray scattering data indicate with high probability, that in an 1 : 1 mixture of enzyme and tRNA in solution an 1 : 1 complex of both components is built up. In this respect the data agree with the results obtained by experiments with the affinity elution system [5]. This is in a certain way contradictionary to the results from a rapid kinetic investigation, which indicated the existence of two independent binding sites [6, 7]. This discrepancy is difficult to explain at the moment, since we do not know very much about the interprotomer interactions in the aminoacyl-tRNA synthetases composed of subunits. In this respect it is of interest however, that the enzyme under certain conditions seems to behave as a dimer with two independent binding sites whereas under other, but closely related, conditions it may exhibit interprotomer catalytic cooperativity as described by Fasiolo *et al.* [10].

Overall shape

In a first step we simply tried to add the tRNA molecule to the model (Fig. 4) which fitted best all data of the free enzyme. To get the experimentally found increase in the radius of gyration the tRNA (approximated by four spheres in a L-shape [24, 25]) had to be placed at the periphery of the model as shown in Fig. 8 (Model 1). But no satisfying agreement could be obtained with all molecular parameters by placing the tRNA at any position to the enzyme.

An information in which way the model is to change to get a better fit can be deduced from the comparison between the two experimental $p(r)$ functions (Fig. 8). The curve of the complex is shifted at the middle region towards smaller distances, while there are a few much larger distances causing the extended tail. This tail is suggested to be caused by the addition of the tRNA molecule in the extension of the longer axis; this would explain the increase in the maximum distance. The shift of the curve of the complex to smaller distances indicates on the other hand that we have to assume a contrac-

tion of the enzyme upon the binding to tRNA. Taking into account these features we set up the Model 2 shown in Fig. 8, which is obtained from Model 1 (simple addition of tRNA) by a contraction of the enzyme molecule by 0.8 nm. This model is in satisfying agreement with all molecular parameters and its $p(r)$ function fits the experimental one well.

We want to point out that the results obtained for the complex are afflicted with a higher uncertainty than those of the free enzyme since we were not able to prove that a completely homodisperse solution of an 1 : 1 complex was investigated. Therefore it is also not meaningful to decide in which position the tRNA is fixed to the enzyme. (The application of the method of contrast variation [26] was not possible since the partial specific volume of enzymes is not the same in the usual buffers and in solutions containing up to 60% sucrose.)

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