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Application of the small-angle X-ray scattering technique for the study of equilibrium enzyme-substrate interactions of phenylalanyl-tRNA synthetase from *E. coli* with $tRNA^{Phe}$

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The small-angle X-ray scattering technique (SAXS) is proposed for the investigation of equilibrium macromolecular interactions of the enzyme-substrate type in solution. Experimental procedures and methods of analysing the data obtained from SAXS have been elaborated. The algorithm for the data analysis allows one to determine the stoichiometric, equilibrium and structural parameters of the enzyme-substrate complexes obtained. The thermodynamic characteristics for the formation of complexes of $tRNA^{Phe}$ with phenylalanyl-tRNA synthetase have been determined and demonstrate negative cooperativity for binding of the two $tRNA^{Phe}$ molecules. The structural parameters (R_g, R_c, semi-axes) have been determined for free phenylalanyl-tRNA synthetase and $tRNA^{Phe}$ from *E. coli* MRE-600 and of enzyme complexes possessing one and two $tRNA^{Phe}$ molecules, indicating structural rearrangements of the enzyme in the interaction with $tRNA^{Phe}$.

Small-angle X-ray scattering; Phenylalanyl-tRNA synthetase; Enzyme-substrate interaction; $tRNA^{Phe}$

1. INTRODUCTION

Small-angle X-ray scattering (SAXS) has been used for investigation of interactions of ligands with macromolecules, namely proteins [1]. SAXS permits one to determine the stoichiometric, equilibrium and structural parameters of the complexes formed by proteins and macromolecular ligands [2–4]. This paper deals with further development of the analysis of SAXS data for equilibrium macromolecular mixtures in solution and its application to investigation of the complexes formed by phenylalanyl-tRNA synthetase with one and two $tRNA^{Phe}$ molecules.

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2. MATERIALS AND METHODS

A homogeneous preparation of phenylalanyl-tRNA synthetase from *E. coli* MRE-600 was obtained as in [5]. $tRNA^{Phe}$ with an acceptor activity of 1100–1500 pmol Phe/A_{260} unit was obtained from Boehringer Mannheim. A buffer consisting of 0.05 M potassium phosphate (pH 7.5), 0.005 M $MgCl_2$ and 20% glycerine was used. X-ray scattering was monitored with a small-angle X-ray diffractometer (Siemens, FRG) [4]. CuK_α radiation (λ = 1.54 Å) was employed. Measurements were taken within the range $0.01322 \leqslant h \leqslant 0.0622$ ($h = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle). The solutions being investigated were placed in a quartz capillary (diameter, 1 mm; thickness of walls, 0.01 mm) for further vacuumization of the small-angle cell. The sample was thermostatted at 20°C. Measurements on each sample were repeated 3–5 times, the mean error at each point being 0.5%. After subtraction of the background, the experimental curves were smoothed and collimation corrections were made according to Svergun and Feigin [6].

3. RESULTS AND DISCUSSION

To determine the stoichiometry of the enzyme-

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substrate complexes the following model for completely cooperative binding of m enzyme (E) molecules with n molecules of substrate (S) was used [4]:

$$mE + nS \overset{K_{mn}}{\rightleftharpoons} E_mS_n \quad (1)$$

The dissociation constant and material balance are expressed by:

$$K = [E]^m[S]^n/[E_mS_n]$$

$$[E_0] = [E] + m[E_mS_n] \quad (2)$$

$$[S_0] = [S] + n[E_mS_n]$$

where [E], [S] and $[E_mS_n]$ denote the equilibrium concentrations of enzyme, substrate and complex, respectively, $[E_0]$ and $[S_0]$ representing the initial values.

SAXS measurements on enzymes, substrates and their mixtures at various concentrations enable one to determine the differential scattering intensity $\Delta I(h)$, which characterizes the effect of formation of a complex [2–4]:

$$\Delta I(h) = I(h) - I_{E_0}(h) - I_{S_0}(h)$$

$$= I(h) - J_E(h)[E_0] - J_s(h)[S_0] \quad (3)$$

where $I_{E_0}(h)$, $I_{S_0}(h)$ and $I(h)$ denote the respective scattering intensities of enzyme, substrate and mixture, $J_E(h)$ and $J_S(h)$ representing the molar intensities of enzyme and substrate. If the angular range of the measured $I(h)$ from free molecules of enzyme, substrate and mixture includes the Guinier region [6], then extrapolation of the scattering intensities to zero angle [$I(0) = I(h)$, $h \to 0$] may be possible and the value of $J(0)$ for a particle will thus be proportional to the square of its effective molecular mass, which allows one to determine the molar scattering intensity of the complex (E_mS_n) from:

$$J_{mn}(0) = \left(m\sqrt{J_E(0)} + n\sqrt{J_S(0)}\right)^2 \quad (4)$$

where

$$J_E(0) = I_{E_0}(0)/[E_0]$$

and

$$J_S(0) = I_{S_0}(0)/[S_0] \quad (5)$$

The parameters m, n and K_{mn} were estimated on a computer using a numerical optimization procedure [7].

The following expression was used as the minimization criterion:

$$R(m,n,K_{mn}) = \frac{2}{p}\sum_{i=1}^{p} |\Delta\tilde{I}_i(0) - \Delta I_i(0)| / (\Delta\tilde{I}_i(0) + \Delta I_i(0)) \quad (6)$$

where p is the number of various mixtures used ($p = 5$). The $\Delta\tilde{I}_i(0)$ values were determined from [2–4]:

$$\Delta\tilde{I}_i(0) = [E_mS_n]_i(J_{mn}(0) - mJ_E(0) - nJ_S(0)) \quad (7)$$

$J_E(0)$ and $J_S(0)$ were found to be equal to 16.8 and 0.85 $imp \cdot s^{-1} \cdot \mu M^{-1}$, respectively. The experimental values of $\Delta I(h)$ determined according to eqn 3 are shown in fig.1. The value of $[E_mS_n]_i$ was estimated from eqn 2 numerically [7].

Optimization of the parameters according to the criterion of eqn 6 showed that the enzyme-substrate complex includes one molecule of enzyme and no more than two $tRNA^{Phe}$ molecules, in agreement with the results obtained in [8–10]. Hence, the process of enzyme-substrate interaction taking into account the statistical effect and the

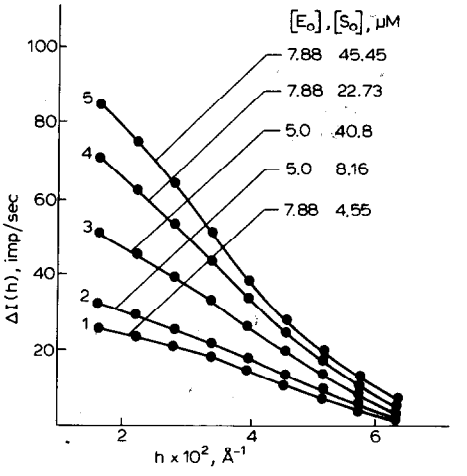

Fig.1. Values of the differential scattering intensity, $\Delta I(h)$, for mixtures of phenylalanyl-tRNA synthetase with $tRNA^{Phe}$ at different initial concentrations of enzyme ($[E_0]$) and $tRNA^{Phe}$ ($[S_0]$). $\Delta I(0)$ values were equal to 28.7 (1), 36.5 (2), 63.8 (3), 82.0 (4), 101.9 (5) imp/s.

phenomenon of cooperativity [8] can be represented by the following scheme:

$$E + S \overset{k/2}{\rightleftharpoons} ES$$
$$ES + S \overset{2k/\varkappa}{\rightleftharpoons} ES_2 \quad (8)$$

where k is the dissociation constant and $\varkappa$ denotes the coefficient of thermodynamic cooperativity.

$$k/2 = [E][S]/[ES]$$
$$2k/\varkappa = [ES][S]/[ES_2] \quad (9)$$

where

$$[E] = [E_0] - [ES] - [ES_2]$$
$$[S] = [S_0] - [ES] - 2[ES_2] \quad (10)$$

The following optimization criterion for estimation of the parameters k and $\varkappa$ was used:

$$T(k,\varkappa) = \frac{2}{p}\sum_{i=1}^{p} |\widetilde{\Delta I}_i(0) - \Delta I_i(0)| / (\widetilde{\Delta I}_i(0) + \Delta I_i(0)) \quad (11)$$

where

$$\widetilde{\Delta I}_i(0) = [ES]_i(J_{ES}(0) - J_E(0) - J_S(0)) + [ES_2]_i(J_{ES_2}(0) - J_E(0) - 2J_S(0))$$

The molar scattering intensities of the complexes, $J_{ES}(0) = 25.2$ and $J_{ES_2}(0) = 35.3$ imp·s^{-1}·μM^{-1}, were estimated from eqn 4. The concentrations $[ES]_i$ and $[ES_2]_i$ were determined from eqn 9 using eqn 10. As a result of optimization the values $k = (0.9 \pm 0.1)$ μM and $\varkappa = 0.10 \pm 0.03$ were obtained. This points to negative cooperativity in the binding of phenylalanyl-tRNA synthetase with tRNA^{Phe}. $J_{ES}(h)$ and $J_{ES_2}(h)$ values were estimated for all combinations of pairs of experimental scattering curves $\Delta I(h)$.

The following system of equations was solved:

$$\begin{cases} [ES]_i J_{ES}(h) + [ES_2]_i J_{ES_2}(h) = \alpha_i(h) \\ [ES]_j J_{ES}(h) + [ES_2]_j J_{ES_2}(h) = \alpha_j(h) \end{cases} \quad (12)$$

where

$$\alpha_{i(j)}(h) = \Delta I_{i(j)}(h) - J_E(h)([ES]_{i(j)} + [ES_2]_{i(j)}) - J_S(h)([ES]_{i(j)} + 2[ES_2]_{i(j)}),$$
$$i(j) = 1,p \ (i = j)$$

As a result, mean values of the molar scattering intensities $J_{ES}(h)$ and $J_{ES_2}(h)$ were obtained (fig.2) which permits one to determine the structural characteristics of the complexes ES and ES_2.

Table 1 lists the structural parameters for macromolecules of the enzyme, tRNA^{Phe} and their complexes: radius of gyration (R_g), radius of gyration of the cross-section (R_c), semi-axes of equivalent ellipsoid rotation (a, b) and the ellipsoid axis ratio (u). These values show that the structure of phenylalanyl-tRNA synthetase from *E. coli* MRE-600 is somewhat different from that of the enzyme obtained from other strains of *E. coli* [9].

The R_g values for complexes containing one and two tRNA molecules differ. The parameter b characterizing the size of the large semi-axis of the ellipsoid of particle rotation for the enzyme complex with one tRNA molecule decreases to 84 Å as compared to 98 Å for the free enzyme. However, on binding of the second tRNA^{Phe} molecule to the enzyme, b increases to 101 Å, which in effect coincides with the size of the large semi-axis of free phenylalanyl-tRNA synthetase. At the same time, the value of the parameter a for the complex with one tRNA molecule increases considerably com-

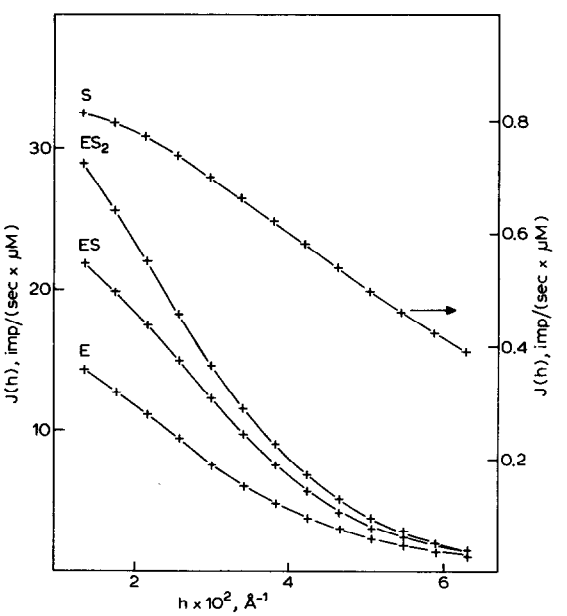

Fig.2. Molar X-ray scattering intensities of the phenylalanyl-tRNA synthetase from *E. coli* MRE-600 (E), tRNA^{Phe} (S) and complexes of the enzyme with one (ES) and two molecules of tRNA^{Phe} (ES_2).

Table 1

Structural parameters for phenylalanyl-tRNA synthetase, $tRNA^{Phe}$ and their complexes, obtained by the SAXS method

Parameter	Enzyme (E)	tRNA (S)	Complex	
			ES	ES_2
R_g (Å)	52.4 ± 0.6	25.6 ± 0.5	50.1 ± 0.8	55.7 ± 2.1
R_c (Å)	28.3 ± 0.4	10.9 ± 0.2	33.1 ± 0.5	32.5 ± 1.0
a (Å)	44.7 ± 1.6	17.2 ± 0.3	52.3 ± 0.8	51.4 ± 1.6
b (Å)	98.6 ± 1.0	51.1 ± 1.0	84.1 ± 1.7	101.0 ± 4.2
u	2.2 ± 0.1	2.96 ± 0.1	1.6 ± 0.2	1.97 ± 0.15

pared to that of the enzyme, however there is no further change in the value for the complex with two tRNA molecules.

The data can therefore reflect conformational adaptation of the enzyme on binding with the tRNA in complexes containing one and two $tRNA^{Phe}$ molecules, in good agreement with the difference found in the thermodynamic characteristics of binding for a two-stage process. The difference in effectiveness of binding of phenylalanyl-tRNA synthetase with one and two $tRNA^{Phe}$ molecules may be assumed to contribute to regulation of enzyme activity in the aminoacylation reaction [10].

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