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# HYDRODYNAMIC PROPERTIES AND STRUCTURE OF THE RAT LIVER 12 S ARGINYL- AND LYSYL-tRNA SYNTHETASE COMPLEX

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Eukaryotic aminoacyl-tRNA synthetases occur in multienzyme complexes in contrast to their prokaryotic counterparts. A core 12 S rat liver complex ($M_r$ 290,000) was recently purified to homogeneity consisting of two polypeptides with $M_r$ 73,000 and 65,000 identified as lysyl- and arginyl-tRNA synthetase, respectively (Dang et al. (1982) Biochemistry 21,1959-1966). Using the modified hydrodynamic theory of Kirkwood (Kirkwood, J.R. (1954) J. Polym. Sci. 12,1-14), we have determined that the model most consistent with the hydrodynamic properties of the 12 S complex is a tetrameric tetrahedral model.

The aminoacyl-tRNA synthetases from higher eukaryotes have recently been conclusively shown to occur in high-$M_r$ multienzyme complexes (1,2). The high-$M_r$ complexes appear ubiquitous in many higher eukaryotes (3-9). With the recent achievements in purifying the complexes, the organization of the high-$M_r$ complexes is now better understood (2); however, the spatial relationship between the synthetases is still not clearly known.

A core 12 S rat liver complex ($M_r$ 290,000) was purified to homogeneity consisting of two polypeptides ($\alpha_2\beta_2$) of $M_r$ 73,000 and 65,000 identified as lysyl- and arginyl-tRNA synthetase, respectively (3). Using the well defined physical characteristics of the 12 S rat liver complex (Table I) in conjunction with hydrodynamic calculations, we demonstrate that the model most consistent with the hydrodynamic properties of the 12 S complex is a tetrameric tetrahedral model. This approach to study the spatial arrangement of the core complex provides a basis for the understanding of the larger high-$M_r$ complexes (2).

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Table I. Summary of Physical Parameters of Arginyl- and Lysyl-tRNA Synthetase Complex

| Property | Method | Value |
|---|---|---|
| Partial specific volume ($\bar{v}$) | Amino Acid and carbohydrate composition | 0.725 cc/g |
| Stokes radius ($R_s$) | Gel filtration | 58 Å |
| Diffusion coefficient ($D_{20,w}$) | Calculated from $R_s$ | $3.5 \times 10^{-7} cm^2/s$ |
| Sedimentation coefficient ($S_{20,w}$) | Sedimentation velocity | 12.1S |
| Subunit molecular weight | SDS gel electrophoresis | 73,000 + 65,000 |
| Native molecular weight ($M_r$) | Calculated from $S_{20,w}$ $R_s$ and $\bar{v}$ | 290,000 |
| Frictional coefficient ratio ($f/f_o$) | Calculated from $R_s$ | 1.15 |
| Axial ratio (a/b) | From $f/f_o$ | 3.5 |

## Results and Discussion

The hydrodynamic properties of complexes composed of identical subunits with different geometric configurations can be estimated by the theory of Kirkwood (10-15). Table II is a tabulation of ratios of sedimentation coefficients and Stokes radii for tetrameric complexes based on three different formulations:

1) Formulation of the Kirkwood theory (10-13).

2) Formulation of Teller et. al. (14).

3) Formulation of Wilson and Bloomfield (15,17).

The Stokes radii and sedimentation coefficients of symmetric proteins are approximated empirically. An empirical equation relating molecular weight to sedimentation coefficient has been determined by Squire and Himmel (16) for 21 relatively symmetric proteins with known crystallographic structure and is given by:

$$M_r = 6850\ S^{3/2} \pm 0.09\ M_r\ (S.D.) \quad (1)$$

We have determined an empirical equation (Equation 2) for 79 proteins the properties of which are obtained from Sober (18). The molecular weight range is 6,669 to 1,015,000 daltons; $S_{20,w}$ range is $1.14 \times 10^{-13}$

Table II. Geometry-dependent Oligomer to Monomer Ratios of Sedimentation Coefficients and Stokes Radii

| Structure | A[1] | | B[2] | | C[3] | |
|---|---|---|---|---|---|---|
| | $S_n/S_1$ | $R_n/R_1$ | $S_n/S_1$ | $R_n/R_1$ | $S_n/S_1$ | $R_n/R_1$ |
| Rod | 2.083 | 1.920 | 2.032 | 1.969 | 1.049 | 2.055 |
| Square | 2.353 | 1.700 | 2.333 | 1.715 | 2.179 | 1.836 |
| Tetrahedron | 2.500 | 1.600 | 2.462 | 1.625 | 2.235 | 1.790 |
| Centered Triangle | 2.183 | 1.832 | N.D | N.D. | 2.015 | 1.987 |

(1) Values in (A) are obtained from and calculated according to van Holde (12).

(2) Values in (B) are obtained from Teller et al. (14).

(3) Values in (C) are obtained from Garcia Bernal and Garcia de la Torre (15).

N.D. signifies not determined.

$S_n/S_1$: Sedimentation coefficient ratio of oligomer to monomer

$R_n/R_1$: Stokes radius ratio of oligomer to monomer

to $26.6 \times 10^{-13}$ s; and $f/f_o < 1.35$. This equation, in excellent agreement with Equation 1, is given by:

$$M_r = 6827\ S^{3/2} \pm 0.05\ M_r\ (S.D.). \quad (2)$$

To approximate the Stokes radius of a protein with a given molecular weight, we have determined an empirical equation (Equation 3) relating molecular weights and Stokes radii derived from diffusion constants of 21 proteins provided by Squire and Himmel (16).

$$M_r = 1700\ R^3 \pm 0.31\ M_r\ (S.D.), \quad (3)$$

where R is the Stokes radius in nanometers. Least square fitting of molecular weights and the cube of Stokes radii of 79 proteins (18) gave the following equation in good agreement with Equation 3:

$$M_r = 1770\ R^3 \pm 0.24\ M_r\ (S.D.) \quad (4)$$

The molecular weight estimate appears to be more reliable from the sedimentation coefficient than from the Stokes radius as shown by the standard deviations of Equations 1-4. The equation of Squire and Himmel (16) is shown here to be applicable to a wide range of sizes of protein as indicated by the similar proportionality constants of Equations 1 and 2.

Table III. Empirical Determination of Sedimentation Coefficient and Stokes Radius of Different Subunit Molecular Weights

| | A | | B | |
|---|---|---|---|---|
| Molecular Weight | $S_{20,w}$ | R(nm) | $S_{20,w}$ | R(nm) |
| 65,000 | 4.48 | 3.37 | 4.49 | 3.32 |
| 73,000 | 4.84 | 3.50 | 4.85 | 3.45 |
| 69,000 | 4.66 | 3.44 | 4.67 | 3.39 |

Values in (A) are calculated from Equations 1 and 3; see text.

Values in (B) are calculated from Equations 2 and 4; see text.

The arginyl- and lysyl-tRNA synthetase complex was shown to exist as a tetramer composed of subunits of two different molecular weights, 73,000 and 65,000 (3). Table III shows the estimated sedimentation coefficient and Stokes radius of the $M_r$ 73,000 and $M_r$ 65,000 subunits. Table III also shows the sedimentation coefficient and Stokes radius of a $M_r$ 69,000 subunit. It can be seen that a subunit of $M_r$ 69,000 is a reasonable estimate for the calculations of the different tetrameric configurations consisting of identical spherical subunits. The calculated sedimentation coefficient and Stokes radius for the lysyl-tRNA synthetase subunit ($M_r$ 73,000) and for the arginyl-tRNA synthetase subunit ($M_r$ 65,000) are not significantly different from the representative $M_r$ 69,000 subunit (Table III) or from the experimental approximate values reported for lysyl-tRNA synthetase (19,20) and arginyl-tRNA synthetase (19,21). Table IV shows the calculated

Table IV. Sedimentation Coefficients and Stokes Radii of Different Tetrameric Configurations*

| | A[1] | | B[1] | | C[1] | |
|---|---|---|---|---|---|---|
| Structure | $S_{20,w}$ | R(nm) | $S_{20,w}$ | R(nm) | $S_{20,w}$ | R(nm) |
| Rod | 9.7 | 6.6 | 9.5 | 6.8 | 9.1 | 7.1 |
| Square | 11.0 | 5.9 | 10.9 | 5.9 | 10.2 | 6.3 |
| Tetrahedron | 11.7 | 5.5 | 11.5 | 5.6 | 10.4 | 6.2 |
| Centered Triangle | 10.2 | 6.3 | - | - | 9.4 | 6.8 |

* A subunit of $M_r$ 69,000 with a sedimentation coefficient of 4.66 S and a Stokes radius of 3.44 nm is used.

(1) Values in (A), (B), and (C) are calculated from (A), (B), and (C) of Table II, respectively.

sedimentation coefficient and Stokes radii for different tetrameric configurations of identical spherical $M_r$ 69,000 subunits with a sedimentation coefficient of 4.66 S and a Stokes radius of 3.4 nm. The approximation using a spherical subunit is substantiated by the occurrence of highly symmetrical subunits in tyrosyl-tRNA synthetase (22) and aspartyl-tRNA synthetase (23) as determined by X-ray crystallography. From the data obtained (Table IV), the structure most consistent with the properties of arginyl- and lysyl-tRNA synthetase complex is the tetrahedral configuration. The square planar configuration, however, cannot be totally ruled out solely by hydrodynamic calculations and must await further structural studies by electron microscopy.

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