ON THE PRESENCE OF A DENSE INTERNAL REGION IN THE 50 S SUBPARTICLE OF *E. COLI* RIBOSOMES

I.N.SERDYUK, N.I.SMIRNOV, O.B.PTITSYN and B.A.FEDOROV
Institute of Protein Research, Academy of Sciences of the USSR, Pushtchino, Moscow Region, USSR

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1. Introduction

An important characteristic of the internal structure of ribosomal subparticles is their density distribution since this may be connected with the relative localization of RNA and protein. We recently showed [1] that the comparison of the electron radius of gyration of compact particles (determined by small-angle X-ray scattering) with their hydrodynamic characteristics allows – in a number of simple cases even quantitatively – the distribution of electron densities of these particles to be estimated. This paper describes the application of the method to *E.coli* ribosomal 50 S subparticles. The radius of gyration, R_g, and the diffusion coefficient, D, were determined on the same preparation of 50 S subparticles.

2. Materials and methods

50 S ribosomal subparticles were obtained from a MRE-600 strain of *E-coli* in Spirin's laboratory at the A.N.Bakh Institute of Biochemistry by a modification of a method described earlier [2] using a B-XV zonal motor of the MSE Superspeed 65 preparative ultracentrifuge. The purity and quality of the preparation was controlled by the Spinco E analytical ultracentrifuge both prior to and after all measurements. The solvent was 10^{-2} M triethanolamine buffer, pH 7.8, containing 10^{-3} M $MgCl_2$ and 10^{-1}M KCl. R_g measurements at all the concentrations (c = 0.3; 0.7 and 1.2%) took one day and D measurements (c = 0.028; 0.041; 0.051 and 0.062%) took three days. As an additional control of stability of the preparation during the course of the experiments, R_g measurements were repeated on the second and third day and gave the same results. R_g was determined at room temperature and D at 22.5°.

The X-ray scattering intensity $I(\theta)$ (2θ – scattering angle) was measured with the Kratky small-angle camera on the Geigerflex assembly with CuKα radiation (λ = 1.54 Å), the anode tube operating at 30 kV and 30 mA. Scattering radiation was registered by a scintillation counter combined with the Rigaku Denki ECP-TS electronic circuit panel. The collimation used satisfied conditions of infinite slit height. R_g was determined by the Guinier method, i.e. from the slope of the curve $\log I$ versus $4\theta^2$ (fig. 1). The scattering curve for every preparation was recorded in the interval 2θ = 6–35 min; 3000 impulses were fixed at every angle. In the indicated interval of the angles the intensity dropped eight-fold, while in the straight line portion of the Guinier plots dropped five-fold.

D was determined using Tsvetkov's polarizing interferometrical diffusometer [3]. In all the experiments, the areas under the refractive index gradient curves $\partial n(x)/\partial x$ remained constant with time. The D value was determined by the standard procedure from the slope of the straight line expressing dependence of the value $1/K = 2\sigma^2$ (σ is the standard deviation of curve $\partial n(x)/\partial x$) versus t (fig. 2).

The translational friction coefficient of ebonite 50 S subparticle models was estimated by measuring their surfacing speed in a cylindrical tank with glycerine. Speeds were used which met the requirements

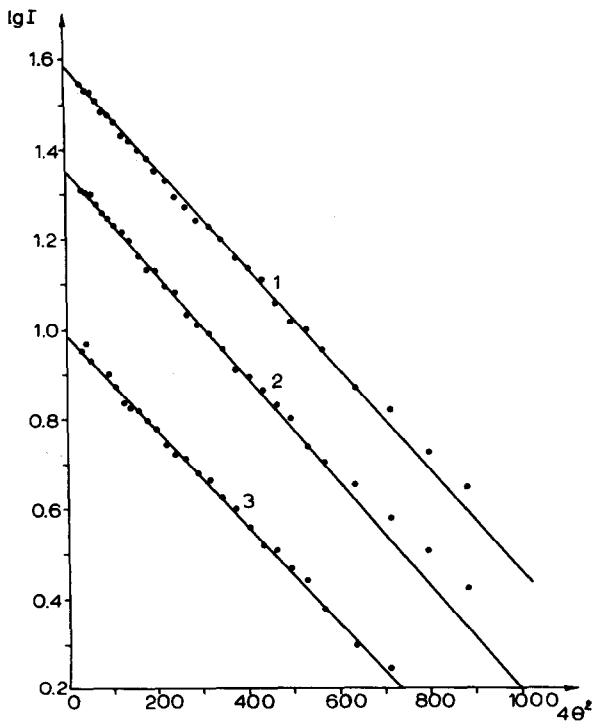

Fig. 1. Dependence of $\log I(\theta)$ versus $4\theta^2$ ($I(\theta)$ in impulses per sec, θ in min) for the *E.coli* 50 S ribosomal subparticle.
1: c = 1.2%, R_g = 74.0 Å;
2: c = 0.7%, R_g = 75.3 Å;
3: c = 0.3%, R_g = 73.2 Å.

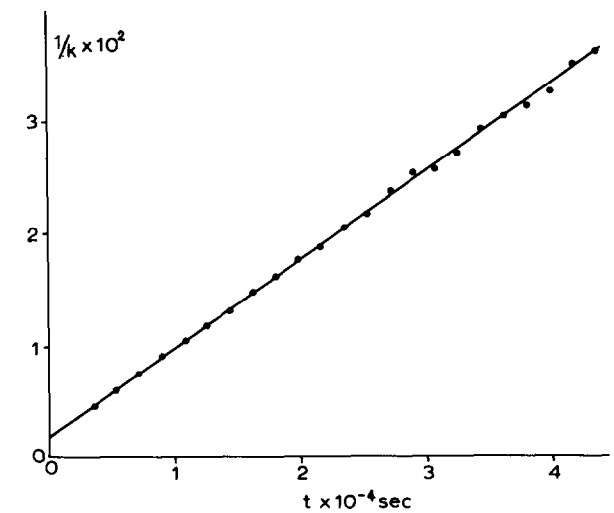

Fig. 2. Determination of the diffusion coefficient. Dependence of $1/K$ versus time t for the *E.coli* 50 S ribosomal subparticle at c = 0.028%.

of Stokes Law for spherical particles and a correction was made for friction of vessel walls.

3. Results and discussion

The values R_g and D estimated from the Guiner plots (fig. 1) and straight lines $1/K$ versus t (fig. 2) do not depend upon concentration, within the limits of error. The value R_g (average of nine measurements) is equal to 74.3 ± 1.0 Å, while the D value (average of four measurements and reduced to standard conditions) gives $D_{20w} = (1.87 \pm 0.04) \times 10^{-7}$ cm^2/sec. The values obtained coincide within the limits of experimental error with the value R_g = 73.0 Å and $D_{20w} = 1.91 \times 10^{-7}$ cm^2/sec obtained earlier [4,5]. In another study [6] a somewhat greater value, R_g = 77.0 Å, was obtained.

For spherical particles of uniform density a simple correlation exists between the radius of gyration and the diffusion coefficient:

$$R_g = (3/5)^{1/2} \times F/6\pi\eta_0 = (3/5)^{1/2} \times kT/6\pi\eta_0 D ,$$

where F is the coefficient of translational friction and η_0 is the solvent viscosity. From this ratio, when $D = (1.87 \pm 0.04) \times 10^{-7}$ cm^2/sec, η_0 = 0.01 poise and T = 293°K, it follows that R_g = 89 ± 2 Å, i.e. the value is 20% higher than the experimental. This value decreases by only 2 Å if the value obtained by Tissières et al. [5] is substituted for D and remains significantly greater than any of the experimental values of R_g.

Thus the *E.coli* ribosomal 50 S subparticles are not spherical bodies with a uniform density.

It was shown [1] that for any prolate or oblate ellipsoid of revolution with a uniform density, $R_g > (3/5)^{1/2} \times kT/6\pi\eta_0 D$. This means that the 50 S subparticle cannot be modelled by an ellipsoid of revolution with a uniform density, as in this case the experimental value of D would lead to R_g = 89 Å. Consequently, the radius of gyration of the 50 S subparticle is too small for a particle of simple shape with the given coefficient of translational friction.

The disagreement may be explained in two ways: first, the 50 S subparticles are of peculiar shape but have uniform density; second, they contain a denser region, thus leading to a decrease of R_g without a

change in the external dimensions of the particle and, consequently, its coefficient of translational friction.

In order to check the first possibility, models of spherical particles – having the smallest R_g at a given F – with hollows on the surface were studied. The number and dimensions of these hollows were selected so as to reduce the radius of gyration with a minimal change of its translational friction coefficient. It was found, however, that the translational friction coefficients of such particles – determined by ebonite models as mentioned above – decreased approximately proportionally to the decrease of the radius of gyration, so that the correlation between R_g and F was practically unchanged. R_g and F were also determined for semi-spherical particle models – with a hollow on the flat surface – like the model of the 50 S subparticle proposed by Bruskov and Kiselev [7]; however, a decrease in R_g/F was not observed in this case either.

These values of R_g and D are incompatible with the first assumption that the 50 S subparticles are of peculiar shape but of uniform density.

This leaves only the second possibility that the 50 S subparticles consist of a denser core and a less dense peripheral part. This could be explained by assuming that the RNA component is located on average nearer to the center of the particle than the protein component.

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References

[1] I.N.Serdyuk and O.B.Ptitsyn, Molek. Biol., in press.
[2] L.P.Gavrilova, D.A.Ivanov and A.S.Spirin, J. Mol. Biol. 16 (1966) 473.
[3] V.N.Tsvetkov, Zh. Eksperim. i Teor. Fiz. 21 (1951) 701.
[4] R.L.Kayushina and L.A.Feigin, Biofizika 14 (1969) 957.
[5] A.Tissières, J.D.Watson, D.Schlessinger and B.R.Hollingworth, J. Mol. Biol. 1 (1959) 221.
[6] W.E.Hill, J.D.Thompson and J.W.Anderegg, J. Mol. Biol. 44 (1969) 89.
[7] V.I.Bruskov and N.A.Kiselev, J. Mol. Biol. 37 (1968) 367.