JOINT USE OF LIGHT, X-RAY AND NEUTRON SCATTERING FOR INVESTIGATION OF RNA AND PROTEIN MUTUAL DISTRIBUTION WITHIN THE 50S SUBPARTICLE OF *E. COLI* RIBOSOMES

I. N. SERDYUK and A. K. GRENADER

Institute of Protein Research, Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR

Received 14 July 1975
Revised version received 17 September 1975

1. Introduction

In *E. coli* ribosomal 50S subparticles the RNA component is on average located nearer to the center of the particle than is the protein component [1]. This conclusion was based on a comparison of the diffusion coefficient of these subparticles with small-angle X-ray scattering data. The aim of this paper is to use the possibilities of a new approach based on the joint use of light, X-ray and neutron scattering to get quantitative parameters characterizing the distribution of RNA and protein in the 50S subparticle. Analysis of the parameters obtained unambiguously indicates the preferential localization of RNA in the center of the 50S subparticle.

2. Materials and methods

It was reported [2] that in a two-component particle the following expression is valid for the radius of gyration R_g determined experimentally from each type of emission:

$$R_g^2 = R_1^2 x_1 + R_2^2(1-x_1) + L^2 x_1(1-x_1) \qquad (1)$$

Here R_1 and R_2 are the radii of gyration of separate components relative to the center of gravity of each, L is the distance between the centers of gravity of the two components, $x_1 = \Delta\rho_1 V_1/(\Delta\rho_1 V_1 + \Delta\rho_2 V_2)$ is the relative scattering fraction of the first component and V_1, V_2 and $\Delta\rho_1$, $\Delta\rho_2$ are volumes and excess scattering capabilities of a volume unit of the first and second components, respectively. As was shown [3] for different classes of two-component biological systems, including nucleoproteins, the $\Delta\rho_1$ values and, consequently, the x_1 values essentially depend on the type of emission used (light, X-rays or neutrons). It follows that by determining the experimental value of the radius of gyration of the ribosomal particle for each type of emission, it is possible to solve type (1) system of equations and to estimate the R_1, R_2 and L values characterizing the mutual distribution of components within the particle.

50S ribosomal subparticles were prepared from a MRE-600 strain of *E. coli* by a modification of the technique described earlier [4] using the B-XV zonal rotor of the MSE Superspeed 65 preparative ultracentrifuge. The 50S subparticles were stored under ammonium sulphate [5]. Measurements were done in a buffer with 0.07 M KCl, 0.01 M Tris–HCl, 1 mM Mg^{2+}, pH 7.1. Analytical centrifugation always showed one homogeneous peak.

X-Ray scattering intensity was measured by a Kratky small-angle camera on a Geigerflex assembly in a special thermostated room. Scattered radiation was registered by a scintillation counter combined with a Rigaku Denki ECP-TS electronic circuit panel and an automatic programmer designed in the Laboratory of Protein Physics at the Institute of Protein Research. Alternate measurements of scattering intensities of solution and solvent in the two capillaries were done using a precision sliding device. After a round of measurements the solution and the solvent were interchanged without changing the geometry of

disposition of the capillaries [6]. Fedorov's program [7] was used in the collimation correction of X-ray curves.

Besides this, measurements of X-ray scattering intensity were made at the University of Wisconsin on a four-slit Beeman camera [8]. Here Lake's program was used in the collimation correction of X-ray curves [9].

Neutron scattering was measured at the Brookhaven National Laboratory with an apparatus described in [10]. Each scattering curve was measured by summing several cycles during 4–8 hr with a scanning interval of 0.07°. The interval of the measured angles (2θ) was 0.42–2.1°. The wave length used was 4.17 Å. The solutions were checked by ultracentrifugation and X-ray scattering before and after neutron scattering measurements. For the ribosome preparations studied the Guinier region was obtained in the interval of 0.42–0.84 Å where the scattering intensity dropped four-times as compared with scattering at the zero angle. In addition to the measurements in a pure H_2O buffer, the intensity was also measured in a D_2O buffer. The relative scattering fraction of the components was calculated on the assumption that for protein and RNA the number of H atoms exchanging with D atoms was, respectively, 16 and 8 per thousand daltons [11,12]. Since the D_2O buffer was prepared from H-salts, the calculated scattering amplitude was corrected using the comparison of its scattering with that of 99.75% D_2O. As the collimator and collector geometry in all the neutron scattering measurements was analogous to that described in [10], the value of 0.8 Å was added to the experimentally measured R_g value to correct for slit smearing.

Measurements of the radius of gyration by light scattering were made with a special apparatus operating on the principle of synchronous summation of intensities and designed at the Institute of Protein Research [13,14]. The identity of radii of gyration values of a single-component particle obtained with the techniques of light and small-angle X-ray diffuse scattering was reported for polystyrene [6]. An analogous comparison of data obtained with neutron and X-ray spectrometers was done by Engelman on RNA-polymerase [15].

For X-ray and neutron scattering, the ρ_j values for each component were calculated in a standard way proceeding from the chemical composition of the component and its partial specific volume $\bar{V}_j$ according to [2].

In the case of light scattering the $\Delta\rho_j$ value was determined from the refractive index increment $(dn/dc)_j$ and the $\bar{V}_j$ value by [2].

The dependence of the relative scattering fraction of the protein component on the volume fraction of protein was estimated for nucleoproteins [3] using 1/4 of a hemoglobin molecule ($C_{738}, H_{1166}, O_{208}, N_{203}$, $\bar{V} = 0.74$ cm^3/g) as a model of the protein component and *E. coli* RNA (21% uracil, 23% cytosine, 31% guanine, 25% adenine, $\bar{V} = 0.55$ cm^3/g) as that of the RNA. For the 50S ribosomal subparticle the following volume values of the protein and RNA were used to estimate the value x_{prot} of the protein component: $V_{prot} = 5.9 \times 10^5$ Å^3, $V_{RNA} = 1.02 \times 10^6$ Å^3, obtained from the known mol. wts of the components ($M_{prot} = 4.80 \times 10^5$ and $M_{RNA} = 1.10 \times 10^6$) and from the partial specific volumes given above. These calculations lead to the following values of x_{prot} for the 50S subparticle: X-rays – 0.19_5, light – 0.31_0, neutrons in H_2O – 0.25_8 and neutrons in D_2O – 0.47_5.

3. Results and discussion

Fig.1 (curves 1, 2, 3) presents the results of measuring the angle intensity of X-ray scattering in

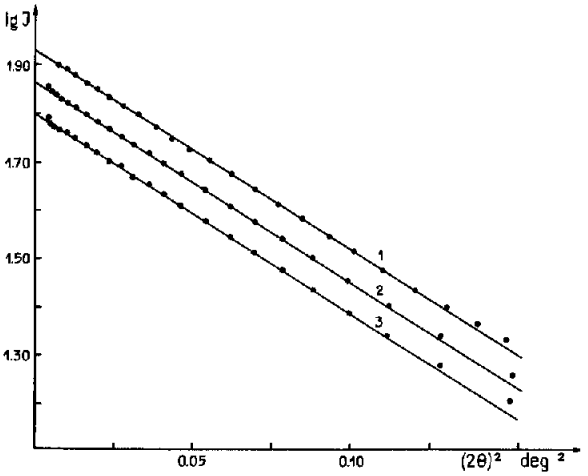

Fig.1. Dependence of the logarithm of the excess intensity of X-ray scattering on the square of the scattering angle; 2θ is the scattering angle in degrees: (1) Kratky camera; (2) Beeman camera, H_2O buffer; (3) Beeman camera, D_2O buffer; in all cases the concentration was ≈ 7 mg/ml.

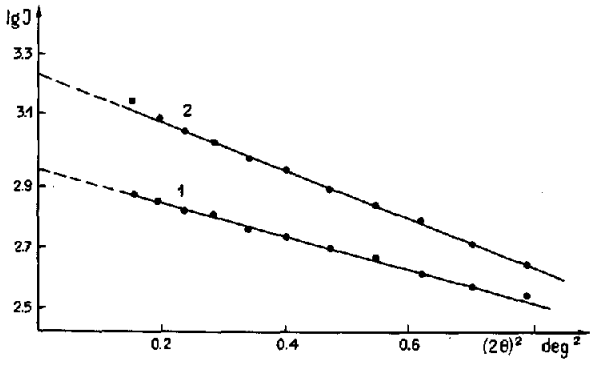

Fig.2. Dependence of the logarithm of the excess intensity of neutron scattering on the square of the scattering angle, 2θ is the scattering angle in degrees. ((1) H_2O buffer (2) D_2O buffer, C=15.5 mg/ml).

the Guinier region. The practical identity of the measured R_g values for the 50S ribosomal subparticles was established by the use of two separate instruments, the Kratky camera (curve 1) and the Beeman camera (curve 2). Extrapolation to zero concentration of the radius of gyration leads to the value of 74.9 ± 0.5 Å. Collimation correction gives practically no increase in the R_g value. The same value of the radius of gyration was obtained using the D_2O buffer (curve 3), indicating that the D_2O per se does not affect the radius of gyration of ribosomes.

However, in the case of neutron scattering, the radii of gyration measured for ribosomes in the H_2O and D_2O buffers differ significantly (fig.2, curves 1 and 2, respectively). The radii of gyration, calculated from these data, are 75.4 ± 1.4 Å and 85.7 ± 1.5 Å (without collimation correction). The dependence of the reciprocal values of light scattering intensities on $\sin^2\theta/2$ (θ is the scattering angle) is a straight line over the entire interval of scattering angles measured (fig.3). The radius of gyration calculated from this is 79.0 ± 1.6 Å. These data show that the experimentally determined radius of gyration for the 50S ribosomal subparticle essentially depends on the type of the emission used. The fact that such a dependence exists itself shows that there is a differential distribution of RNA and protein in the 50S subparticle. The character of this distribution can be established by formula (1), plotting the dependence of the square of the measured radius of gyration versus the relative scattering fraction of one of the components (fig.4). This shows that the

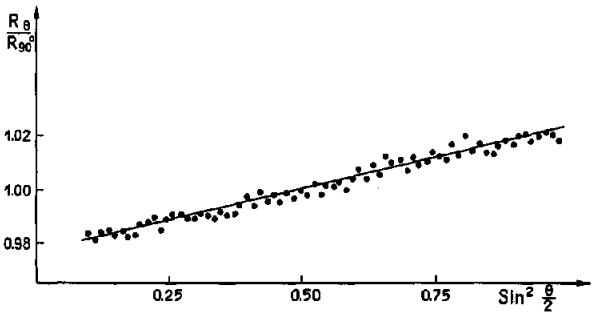

Fig.3. Dependence of the reciprocal value of light scattering intensity on $\sin^2\theta/2$ (θ is the scattering angle at λ=366 nm and C=0.16 mg/ml). Every eighth point is shown as the average of the eight neighbouring points.

R_g^2 value increases with an increase in the relative scattering fraction of the protein component, i.e. the R_g of the protein is greater than that of the RNA. At this relationship between the radii of gyration of the components and the relatively small experimental interval of changing the $x_1(1-x_1)$ coefficient at L, estimation of the L value from our data is impossible. However, neglect of the L values does not introduce significant corrections to the estimated R_g values of RNA and protein. Theoretical analysis by formula (1) shows that in our conditions the absolute R_g values of the components are practically independent of L on changing the L value in wide limits from 0 to 50 Å, and the R_g ratio of the components does not practi-

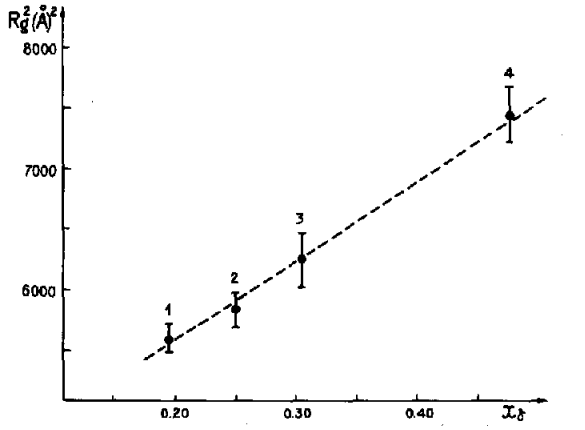

Fig.4. Dependence of the square of the experimental radius of gyration for three types of emission on the relative scattering fraction of the protein component. (1) X-rays, (2) neutrons in H_2O, (3) light scattering, (4) neutrons in D_2O.

cally change when L varies from 0 to 100 Å. Calculation of the protein R_g gives the value of 104 ± 3 Å, and that of RNA 65 ± 2 Å*.

4. Conclusion

The values of the RNA and protein radius of gyration obtained in these studies corroborate the conclusion reported earlier [1] that on average the RNA is nearer to the center of the particle than is the protein. (It should be noted for comparison that the minimal R_g value of the RNA corresponding to its dense packing as a sphere in the center of the 50S subparticle is 49 Å.) Moreover, such a great difference in the radii of gyration of RNA and protein implies a definite scheme of mutual RNA and protein arrangement in the 50S subparticle – namely the distribution of the greater mass of proteins on the RNA surface.

Acknowledgements

One of the authors (I.N.S.) would like to express his sincere gratitude to Professor J. Anderegg (University of Wisconsin, Madison, USA) for providing the possibility of working in his laboratory, for the attention and interest in the experimental work, to Dr B. Schoenborn (Brookhaven National Laboratory, New York, USA) for the possibility to carry out neutron scattering measurements and to Dr D. Engelman (Yale University, New Haven, USA) for help in these measurements. Dr I. N. Serdyuk expresses his gratitude to Ch. Trumball (USA National Academy of Sciences) for the excellent organization of his scientific work in the USA. The authors thank Professor A. S. Spirin and Professor O. B. Ptitsyn (Institute of Protein Research, Academy of Sciences of the USSR) for critical comments and interest in the work. The present research was carried out within the framework of the USSR–USA Intergovernment Agreement on Scientific Exchange.

* The significant differences in the RNA and protein radii of gyration in the 50S subparticle reported here are in evident contradiction with the data of Moore, Engelman and Schoenborn [10] which showed that these values are practically the same. At present it is difficult to show unambiguously the reason of such differences.

References

[1] Serdyuk, I. N., Smirnov, N. I., Ptitsyn, O. B. and Fedorov, B. A. (1970) FEBS Lett. 9, 324–326.
[2] Serdyuk, I. N. and Fedorov, B. A. (1973) J. Pol. Sci. (Polymer Letters) 11, 645–647.
[3] Serdyuk, I. N. (1974) Dokl. Akad. Nauk SSSR 217, 231–234.
[4] Gavrilova, L. P., Ivanov, D. A. and Spirin, A. S. (1966) J. Mol. Biol. 16, 473–489.
[5] Gavrilova, L. P. and Spirin, A. S. (1974) in: Methods in Enzymology, Part I (K. Moldave and L. Grossman eds.), 30, 452–462, Academic Press, New York and London.
[6] Serdyuk, I. N., Polubesov, G. S. and Grenader, A. K., in preparation.
[7] Fedorov, B. A. (1968) Kristallografiya 13, 763–769.
[8] Tolbert, W. R. (1971) Thesis, University of Wisconsin, USA.
[9] Lake, I. A. (1967) Acta Cryst. 23, 191–197.
[10] Moore, P. B., Engelman, D. M. and Schoenborn, B. P. (1974) Proc. Nat. Acad. Sci. U.S. 71, 172–176.
[11] Edelstein, S. I. and Schachman, H. K. (1973) in: Methods in Enzymology, Part D (C. H. W. Hirs and Serge N. Timasheff eds.), Academic Press, New York and London, 27, 82–98.
[12] Page, L. A., Englander, S. W. and Simpson, M. V. (1967) Biochemistry 6, 968–977.
[13] Serdyuk, I. N., Grenader, A. K., Kornilov, V. V. and Sedov, A. I., Vysokomol. Soed. (USSR) in the press.
[14] Serdyuk, I. N. and Grenader, A. K. (1974) Makromol. Chem. 175, 1881–1892.
[15] Engelman, D., private communication.