A SMALL-ANGLE X-RAY SCATTERING INVESTIGATION ON THE STRUCTURE OF THE RNA FROM BACTERIOPHAGE MS2

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1. Introduction

Up to now, only limited information is available on the complete three-dimensional structure of high molecular weight RNA. What we do already know belongs mainly to the primary and secondary structure level. There is also some information about the structure of RNA within certain virus particles, but comparably less is known about the tertiary structure of free RNA molecules in solution. Light scattering and hydrodynamic investigations have however shown that at least some high molecular weight RNAs must have a fairly compact tertiary structure at not too low ionic strength.

Among the best studied high molecular weight RNAs is the genome of the *E. Coli* phage MS2 [1–6]. A sample of this RNA was investigated by small-angle X-ray scattering at ionic strength 0.1. As a result of this study, we are now able to present for the first time detailed data on the overall shape and size of a high molecular weight RNA molecule in the free state*. The shape of MS2 RNA as derived from our scattering data appears to be a both flat and elongate coil of about 620 Å diameter and a ratio of about 2 : 1 : 0.5 for the mean radii of gyration in the three directions of space.

* Some preliminary data were already presented to the Third International Conference on X-Ray and Neutron Small-Angle Scattering (abstract in [7]) and to the Biochemie-Herbsttagung 1973 (abstract in [8]).

2. Materials and methods

The growth and purification of bacteriophage MS2 and subsequent isolation of viral RNA have been described previously [9,10]. Special attention has been paid to the careful sterilization of all glassware and solvents in order to avoid degradation of the RNA by RNAse as much as possible.

All solutions used for X-ray scattering and density measurements have been extensively dialyzed against the following buffer : 0.1 M NaCl, 1 mM Na_2HPO_4, 1 mM NaH_2PO_4, 0.5 mM $MgCl_2$ (pH 6.8). The RNA concentrations have been calculated from optical density measurements at 260 nm, using an optical density of 22.6 for a 1 mg/ml solution at 260 nm and 1 cm light-path.

The X-ray scattering investigations were performed using the techniques and the theory and evaluation procedures described previously [11–16]. The experiments were carried out at 5°C with solutions of the RNA in concentrations of between 3 and 21 mg/ml. The measurements covered an angular range extending from 0.5 to 120 mrad. The data obtained therefrom were finally extrapolated to zero concentration.

3. Results and discussion

The scattering curve of MS2 RNA corresponds in its inner part to that for an anisometric particle (fig.1). The following molecular parameters could be derived therefrom:

(1) The intensity scattered at zero angle furnished a molecular mass of 1.09×10^6 daltons. In this determination, a value of 0.457 cm^3/g as obtained from density measurements with the digital density meter [17] was used for the isopotential specific volume [18,19].

(2) The overall radius of gyration R of the RNA was obtained as 171 Å from the course of the scattering curve at the smallest measured angles and as 181 Å from the correlation function, [20,21] respectively. By comparison with model curves, the latter value is more representative.

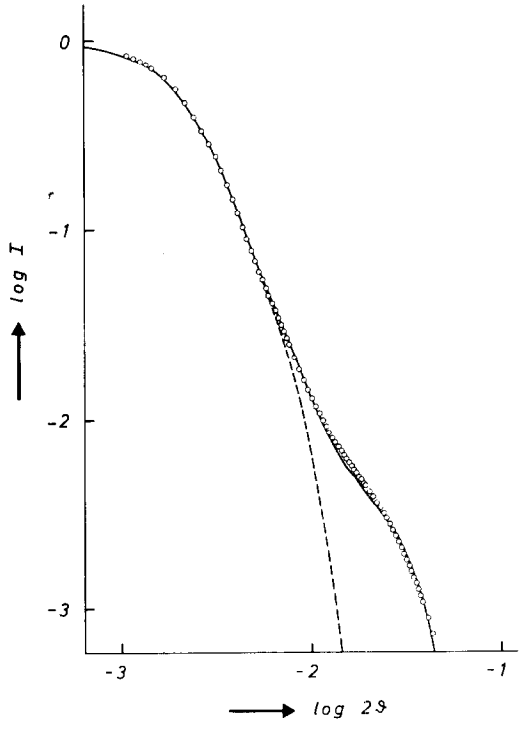

Fig. 1. Plot of log I (2 ϑ) versus log 2 ϑ of the scattering curve for MS2 RNA in phosphate buffer (pH 6.8) containing 0.1 M NaCl and 0.5 mM $MgCl_2$ (o—o—o), and comparison with theoretical curves for models. Dashed curve: oblate elliptic cylinder, 2a = 618 A, 2b = 319 A, H = 94 A; solid curve: coil model, R_x = 158 A, R_y = 77 A, R_z = 40 A.

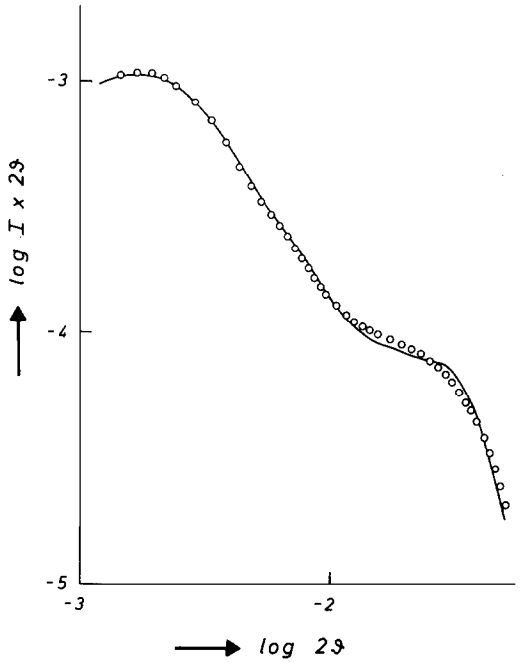

Fig. 2. Plot of log I × 2 ϑ versus log 2 ϑ of the scattering curve for MS2 RNA (o—o—o), demonstrating the two cross-section regions, and comparison with the theoretical curve for the coil model (solid curve).

(3) The largest diameter of the RNA particle is 620 Å, as derived from the correlation function.

(4) At angles somewhat larger than those evaluated for the determination of the overall radius of gyration, the scattering curve is typical for an elongate particle. From this region (see also fig.2) we obtained a mass per unit length of M_c = 1890 daltons/Å and a radius of gyration of the molecular cross-section of R_c = 84.3 Å.

(5) In the angular range from 25 to 50 mrad, the scattering curve is typical for rod-like particles again (fig.2), corresponding to a cross-section radius of gyration of R_{c2} = 9.1 Å and to a mass per unit length of M_{c2} = 169 daltons/Å.

(6) In the angular range just between the two regions typical for elongate particles, the scattering curve resembles that for a flat particle. From this range, a thickness radius of gyration R_t of primarily about 27 Å could be estimated. In the second step of evaluation when the influence of the 25 to 50 mrad region was explicitly taken into account (see below), this radius of gyration was re-estimated as about 40 Å.

Both the molecular weight and the overall radius of gyration as found by us stand in very good agreement with previous results from light scattering investigations with MS2 RNA and the RNA from the closely related phage R17 [1,6,22].

The evaluation and analysis of our results was performed in two steps. In the first step, use was made of the relations between R, R_c and R_t and the radii of gyration R_x, R_y and R_z in the three directions of space. Thus R_z was assumed to equal R_t; R_y was calculated from $\sqrt{R_c^2-R_t^2}$ as 79.9 Å and R_x from $\sqrt{R^2-R_c^2}$ as 154.5 Å. Then from these the absolute axial dimensions were calculated for various homogeneous geometrical bodies. The bodies taken into consideration were prisms, ellipsoids and elliptic cylinders. There are several criteria for proving a model acceptable. One is a satisfying agreement between the values for the particle length as calculated from R_x and from the ratio of M to M_c, respectively. Another criterion is the largest diameter. The value derived from the three dimensions, length, breadth and thickness should agree with the experimental value of 620 Å, which in turn is the only absolute dimension of the particle that can be obtained from its scattering curve without any assumptions on the shape of the particle. On the basis of these criteria, an oblate elliptic cylinder with axes of 618, 319 and 94 Å was found to fit best.

The theoretical curve for this cylinder is also shown in fig.1. The curve fits the experimental curve very well in its inner part, while beginning at about 6 mrad there is a clear discrepancy between the tail ends. This can easily be understood. The theoretical curve refers to a completely homogeneous body, whereas the MS2 RNA particle is surely not homogeneous but exhibits a remarkable substructure that is manifested in the scattering curve mainly in the region from 25 mrad upwards. Obviously minor contributions do occur also at smaller angles and these interfere with the tail end of the scattering curve due to the overall shape of the RNA particle. Consequently, in order to approximate the experimental scattering curve for MS2 RNA by model curves over a much more extended angular range, the models must fit not only the overall shape of the RNA molecule but they must additionally simulate the principal features of its substructure also. The analysis of the substructure and the establishment of appropriate models was the second step of evaluation.

According to our data from the large scattering angles, the substructure of the RNA must consist of extended rod-like regions. Most of them are probably double-helical. However, there must also be a considerable amount of single-stranded, i.e. not base-paired regions, because the experimentally found mass per unit length of 169 daltons/Å is just between the theoretical value of 252 daltons/Å to be expected for an 11-fold RNA double helix [23] and the value of 91 daltons/Å obtained for the RNA in formaldehyde solution at 20°C [8]. The second cross-section radius of gyration of 9.1 Å may refer to double-helical and not base-paired regions as well. These results are in good qualitative agreement with previous observations made with other high molecular weight RNAs [24–26].

To take account of this substructure in the course of model computations, spheres were arranged to linear segments of different lengths whereby appropriate weighting factors were applied to simulate single-stranded and double-helical segments. These segments were linked together and arranged within a given geometrical shape in such a way that no mutual penetrations of segments did occur. The radius of the spheres constituting the segments was chosen in accordance with the experimental value for R_{c2}. The total length of all segments was adjusted according to the experimental M/M_c ratio. The construction of these coil models was facilitated by a computer program which generated the coordinates of the spheres on the basis of random number configurations and a given set of geometrical parameters. From the coordinates, the scattering curves of the models were calculated by means of another computer program [27].

While the first coil models calculated were still based on the oblate elliptic cylinder derived in the first step of evaluation, it soon turned out advantageous to increase the thickness of the models in order to achieve a better curve fit. Consequently R_t increased also. In the final model it amounts to about 40 Å.

A particle of the kind of MS2 RNA, filling only about 10% of the volume defined by its overall shape, surely cannot be completely rigid but must show some flexibility. This was taken into account in that the final model curve (fig.1) was obtained by averaging over the curves calculated for several models of similar but not identical dimensions. These models corresponded to a double-helix content of about 72% [28].

As the comparison in fig.1 demonstrates, the coil model curve can be considered as a sufficient approximation to the scattering curve of MS2 RNA.

The mean radii of gyration of the model in the three directions of space are R_x = 158 Å, R_y = 77 Å and $R_z = R_t$ = 40 Å. From these, the overall radius of gyration follows as R = 180 Å and the cross-section radius of gyration as R_c = 87 Å. Thus with the exception of R_t, almost the same radii of gyration as before have been obtained. It should however be noted that even on this stage the value for R_t is less accurate than the other parameters.

If from R_x, R_y and R_z the absolute dimensions would be calculated for a homogeneous elliptic cylinder, the lengths of the axes would result as 632, 306 and 140 Å. These are very similar to the dimensions of the cylinders that were chosen as limiting shape of the coils in the course of model building.

4. Conclusion

As derived above the shape of native MS2 RNA can be described by a coil of about 620 Å diameter and a ratio of about 2 : 1 : 0.5 for the radii of gyration in the three directions of space. With respect to polydispersity of size and shape, the above dimensions may be regarded as mean values that define a body equivalent in scattering to the average structure of MS2 RNA.

The model obtained by us is contrary to the model previously derived from light scattering and hydrodynamic measurements [6]. That model corresponds to a diameter of about 900 Å and a cross-section radius of gyration R_c of about 50 Å. These dimensions are incompatible with our experimental data.

The discrepancy between the two models may have several reasons. The derivation of a model from a combination of hydrodynamic and light scattering data as done by Slegers et al. is based on the assumption that the hydrodynamic volume and axial ratio are the same under the conditions of the three different measurements. It is questionable whether this assumption still holds for a coil such as MS2 RNA. Furthermore, the hydrodynamic methods can describe a particle only by an equivalent ellipsoid of revolution. The actual shape of the particle may differ considerably from this ellipsoid. As compared to the hydrodynamic methods, small-angle X-ray scattering furnishes more direct and more detailed information about the shape of a particle. Therefore the model for MS2 RNA as derived from X-ray scattering will certainly match the actual shape of the particle better than the hydrodynamic model can.

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