A NEUTRON STUDY OF THE 30 S-RIBOSOME SUBUNIT AND OF THE 30 S-IF3 COMPLEX

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ABSTRACT :

Neutron small angle scattering has been used to study the 30 S-ribosome subunit and the complex of this subunit with the factor IF3. Contrary to previous report we do not observe a change of the conformation of the subunit induced by IF3. The radius of gyration of the 30 S is found to vary with the D_2O content of the solvent. This implies that the RNA has a radius of gyration smaller than the protein (62 Å for the RNA, 80 Å for the protein and 69.5 Å for the subunit in H_2O).

The factor IF3 is known to play a key role in the attachment of a m RNA to the 30 S ribosomal subunit, (1,2) ; it also inhibits the association of the 30 S and 50 S subunits (3-6). A simple model to explain both of these functions has recently been proposed (7). It has been reported (8) that the interaction of IF3 with the 30 S subunit induces a very large change of conformation of that subunit. On the basis of hydrodynamical studies and X-rays small angle scattering the authors have claimed that the 30 S-IF3 complex has a nearly spherical shape (92.5 x 138.5 x 138.5 $Å^3$) compared with the 30 S alone (60 x 220 x 220 $Å^3$). In order to understand the mechanism of the action of IF3 we thought useful to reinvestigate that question using neutron small angle scattering. The advantage of that technique is that it provides information not only on the conformation of the subunit but also on the conformation of the two moieties (9,10). Our experiment does not confirm the existence of a change of configuration of the 30 S subunit induced by IF3 ; it also gives

some indication on the relative distribution of the RNA and of the proteins in that subunit.

MATERIALS AND METHODS

The 30 S subunit and the IF3 were prepared as described earlier (12). The measurement in one series was done by first collecting data on the 30 S and then adding the IF3 factor in the measuring cell and collecting a new set of data (Protocol A). In another series we collected data on two independent samples, one of 30 S and the other of a stochiometric complex previously assessed by radioactivity (Protocol B). IF3 was an homogeneous preparation (one band on an SDS gel electrophoresis (6)).

Protocol A - The buffer contains 5 m M Mg Acetate, 10 m M Tris-HCl (pH 7.4), 50 m M ammonium chloride, 4 μ M or 8 μ M of 30 S ribosome and 4 μ M or 8 μ M of IF3. The water of that buffer was either normal water or a mixture of H_2O and D_2O.

Protocol B - In order to determine the molar ratio of the two components in the complex we used ^{14}C-CH_3IF_3 prepared as described (7) - 30 S subunits (2.8 μ M) were incubated for 15 min at 37°C in 8 m M $MgCl_2$, 100 m M NH_4Cl, 50 m M Tris-HCl (pH 7.5) in absence of IF3 or in presence of 3 times molar excess of IF3 compared to 30 S (containing 4 % of ^{14}C-CH_3 IF3, 4000 cpm/μg). The incubation mixtures were layered on top of 8 ml 10 % sucrose, 8 m M $MgCl_2$, 100 m M NH_4Cl, 50 m M Tris-HCl pH 7.5 and centrifuged for 13 h at 4000 rpm. The pellets were resuspended in the same buffer to a final concentration of 8-9 μ M. By this procedure the complex is separated from free IF3 and we find that each 30 S subunits binds one molecule of IF3.
Data were collected on the neutron small angle camera at the Institut Laue-Langevin (13) using a sample to detector distance of 1055 cm and wavelength of 8 Å. Some data were also collected at 255 cm with 10 Å. Concentration used were in the range 2 to 7 mg/ml.

RESULTS

Figure (1) shows a Guinier plot of data collected in H_2O. Within experimental errors the same radius of gyration is found for the subunit and the 30 S-IF3. Same results were found with the two methods used to study the complex (see Materials and Methods). Similar observations have been done with sample with various D_2O content in the buffer. Figure (2) shows the data measured at larger scattering angle for the 30 S and the 30 S-IF3 complex. Here also no indication is found of a change of configuration. A change of configuration as reported (8), would be seen in a spectacular way, as shown in the figure.
So our data do not support at all previous conclusion of Paradies et al. (8). Indeed, if anything happens, this may be a very slight increase in the radius

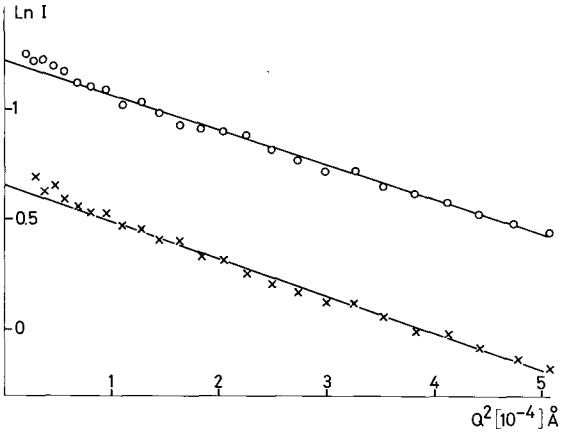

Fig. 1 Guinier plot for the 30 S subunit (-O-) (C - 7 mg/ml) and the 30 S-IF3 complex (-x-) (C = 3.5 mg/ml). The abscissa are in Q^2 where $Q = \frac{2\pi\ \theta}{\lambda}$ where θ is the scattering angle, and λ the wavelength (8 Å)... The samples are in buffer with H_2O and were prepared according to protocol A. The data show some aggregations of the particle (points above the Guinier plot at the origin).

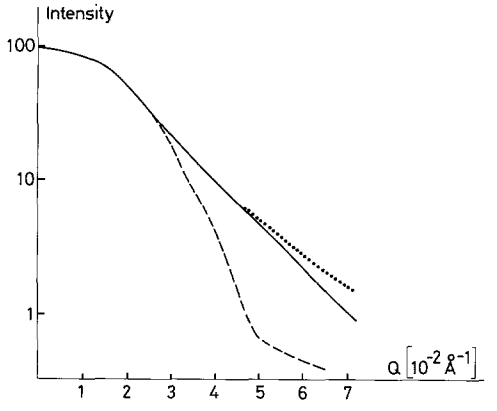

Fig. 2 Intensity versus Q for the 30 S and the 30 S + IF3 complex (prepared following protocol B). (solid curve). The data of both samples are indistinguishable. The buffer is made with H_2O.
... Model calculation for an oblate ellipsoid with axial ratio 1 : 4 : 4
--- Model calculation for an oblate ellipsoid with axial ratio 1 : 1.5 : 1.5. This corresponds to the shape reported by Paradies et al. (8) for the 30 S-IF3 complex.

of gyration, by about 1 %. But this effect is at the limit of experimental errors. Moreover, the samples always show some aggregation in the presence of

IF3, and this may lead to some slight overestimation of R_G. In conclusion we can say that IF3 does not induce a change of configuration of the 30 S subunit.

STUDY IN SOLVENT WITH D_2O

In all cases, we have observed some increase of the aggregation in the presence of D_2O. The importance of that effect varies from sample to sample, and is so far unexplained. This effect is not too large and allows reasonably accurate measurement.

The validity of the measurements is confirmed by two observations :

a) The variation of the square root on the intensity at the origin varies linearly with the D_2O content as it should (14). The intensity vanishes for a solvent with 55 % D_2O. If one uses the values given in (15) for the chemical composition of the 30 S subunit, one can then deduce the dry volume of the subunit by writing that its scattering amplitude is the same as that of the solvent occupying its volume. A volume of $9.5\ 10^5$ $\mathring{A}^3$ is deduced. This corresponds to a specific volume of 0.65, if one uses a molecular weight of 0.87×10^6 daltons.

b) The absolute value of the intensity at the origin can be calculated from the chemical composition and the concentration of the sample. This value can also be measured with appropriate calibration. The two values agree within 10 %. This proves that the extrapolation at the origin is correct, and gives confidence in the values obtained for the radius of gyration.

Typical values obtained for R_G, are listed in table I. The values in H_2O are in very good agreement with those obtained with X-rays (16). This is expected as the relative weight of RNA and protein are rather similar for X-rays and neutron in H_2O. The variation of R_G^2 with the contrast between solvent and the subunit, is shown in figure 3. The same curve is obtained for the 30 S-IF3 complex. From that curve one can deduce a lack of homogeneity of the distribution of the RNA and of the protein. To be quantitative, one can deduce

Table I

% D_2O	R_G (This work)	Ref. (15)	X-rays Ref. (8)	X-rays Ref. (16)
0	69.5 Å	71.4	67.8	69
33 %	67.5			
65 %	80			
99 %	75			

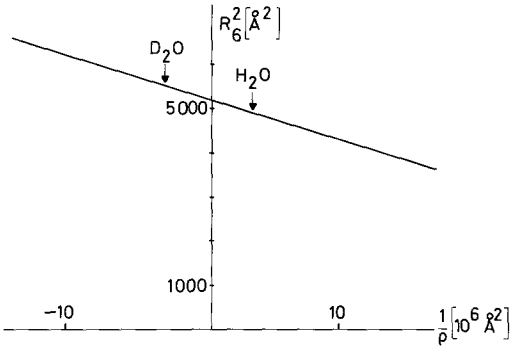

Fig. 3 Variation of R_G^2 with $\frac{1}{\rho}$ where ρ is the contrast of the 30 S with the solvent. This gives a linear plot in agreement with (15).

from the linear plot of figure (3) radius of gyration of both moieties of the 30 S. It is known (11,14) that the scattering density of a protein is matched with a solvent with about 42 % D_2O. With 65 % the RNA is nearly completely matched. Bearing in mind that due to fluctuations in the scattering densities, the matching of a component by the solvent does not mean that the contribution of that component to R_G is absolutely zero, one can give the following figures.

Radius of gyration dominated by the protein : 80 Å

Radius of gyration dominated by the RNA : 62 Å

Those values imply that the center of 30 S is richer in RNA than the border. But this is much smaller than in the 50 S subunit (10). If one combines those values with the weight of both moieties (in physical weight, and in scattering densities), one deduces a value of 69 Å for the R_G of the 30 S in vacuum, very close to the value measured in H_2O. This shows that within experimental errors the center of gravity of both moieties do coincide.

DISCUSSION

Our results, both in H_2O and with various D_2O content in the solvent do not indicate the variation of conformation previously reported (8). In our experiment, we have used stochiometric ratio of IF3 to 30 S, whereas Paradies et al. (8) have used ratio as high as 10 . Aggregation may be a severe problem in dealing with the 30 S. But as mentioned, as our data are collected on absolute scale, we can be fairly sure that we are dealing with the correct Guinier plot. Otherwise, we would observe an incorrect intensity at the origin. Our result agrees with the observation that cross linking in the 30 S is not modified by IF3 (17). Concerning our data on the 30 S itself, they are difficult to compare with those previously obtained with sample selectively deuteriated (15). There is no contradiction between our data and those data. Stuhrmann (Private communication) has also collected data on the 30 S which are in fair agreement with ours. The 30 S subunit appears to be much more homogeneous than the 50 S subunit, but certainly not completely homogeneous.

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