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HYDRODYNAMIC STUDIES ON THE ESCHERICHIA COLI 30S RIBOSOMAL SUBUNITS AND 30S·IF-3 COMPLEX

by

Lallan GIRI, Cynthia L. PON and Claudio GUALERZI

Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem

and

Wolfgang DOSTER and Benno HESS

Max-Planck-Institut für Ernährungsphysiologie, Dortmund, Germany

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Summary: Sedimentation, viscosity and laser light scattering have been used to compare the 30S ribosomal subunits and the 30S·IF-3 complex from Escherichia coli. Contrary to a previous report (1), no detectable change in the hydrodynamic parameters of the 30S subunits was found upon binding of IF-3.

Several lines of evidence support the notion that the conformation of the 30S ribosomal subunit is changed upon IF-3 binding (2-7). Thus all the various activities attributed to IF-3 can be explained with a unitary mechanism simply assuming that the various functional tests of IF-3 activity reflect the newly acquired physico-chemical properties of the 30S·IF-3 complex. The main questions remaining unanswered, however, concern the overall extent of the conformational change and the proportion of the 30S components becoming involved in the change (i.e. whether the change is local or generalized). Concerning this second aspect, the combined data of two different chemical modification techniques, showing that approximately half of the 30S ribosomal proteins acquire different reactivity (enhanced or decreased) (4,5), seem to favor the premise that the change is generalized. A similar conclusion can also be drawn from the observation that separate functional sites of the 30S ribosome (i.e. mRNA (6,7), aminoacyl-tRNA (6,7), 50S (8-11) and IF-1 (12,13) binding sites) are affected by the binding of the factor. With respect to the extent of the change, however, the situation appears to be less clear. In fact, while Paradies et al. (1) claimed to have seen large differences in the hydrodynamic properties and low angle X-ray scattering patterns between 30S and 30S·IF-3 complexes, no major differences were found by neutron scattering (14).

Therefore, in the present paper we have re-examined the hydrodynamic properties of the 30S ribosomal subunits with or without bound IF-3. In

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addition, quasi-elastic light scattering has been employed to ascertain whether a conformational change could be detected by this technique which seems to be particularly suitable to yield information on the size and shape of the ribosomal particle.

Materials and Methods

Escherichia coli MRE 600 30S ribosomal subunits and initiation factor IF-3 were prepared as previously described (15,16). For the preparation of 30S ribosomes and 30S·IF-3 complex, 2200 A_{260} units of 30S ribosomes in Buffer A (Tris-HCl, pH 7.8, 10 mM; Mg acetate, 10 mM; NH_4Cl, 60 mM; 2-mercaptoethanol, 6 mM) were heat-reactivated at 50°C for 5 min and the sample was divided into two 10 ml aliquots which were incubated at 37°C for 15 min following addition of 2.5 mg IF-3 in 0.9 ml of Buffer A (20 mM Tris-HCl (pH 7.7) containing 1 mM EDTA; 200 mM NH_4Cl; 1 mM DTT and 10% glycerol) or 0.9 ml of the same buffer solution. The samples were then loaded onto a total of 12 5-20% sucrose gradients in 20 mM Tris-HCl, pH 7.7; 10 mM Mg acetate; 100 mM NH_4Cl; 0.5 mM DTT and centrifuged for 12 1/2 hrs at 20 K rpm in a SW27 rotor. The fractions corresponding to 30S and 30S·IF-3 complex were pooled and the ribosomes collected by centrifugation. The 30S·IF-3 complexes prepared in this way contained approximately 0.7 molecules of IF-3 per 30S particle determined as previously described (13).

Hydrodynamic studies

The sedimentation velocity experiments were performed in a Beckman Model E ultracentrifuge at 20°C using Schlieren Optics. The apparent sedimentation coefficients were measured at several 30S subunits or 30S·IF-3 concentrations and extrapolated to zero concentration to obtain $S^{\circ}_{20,w}$ values.

The apparent specific volumes were determined from density data of 30S subunits or 30S·IF-3 complex. To measure the density, a precision digital density meter (DMA 60 and DMA 601M, Paar KG, Graz) at a temperature of 20± 0.01°C was used. Values of apparent specific volume were determined from the slope/density increment $\partial\rho/\partial c$ of a linear plot according to the equation (17) $\phi^* = (1 - \partial\rho/\partial c)/\rho_o$ where ρ_o is the density of the solvent. The concentrations were determined spectrophotometrically assuming that there was no significant change in extinction coefficient of 30S subunits after IF-3 binding.

To determine intrinsic viscosity, the relative viscosity was measured at several sample concentrations using an Ostwald-type microcapillary viscometer coupled to an automatic viscosity measuring system (A VS/G, Schott Glass Co., Mainz, FRG). The viscometer had a flow rate of 300 sec for water at 20± 0.05°C. The dilutions of the samples were made in the viscometer and an aliquot was removed before each dilution to be used for density and concentration determination as described above.

Quasi-elastic light scattering

The light source was a 50 mW HeNe laser (Spectra Physics Mod. 125A). The correlation function was obtained from a Malvern digital correlation spectrometer (system 4300) with a temperature controlled specimen cell. The measurements were carried out at θ = 90-110°C and at a temperature of 20°C.

The normalized intensity autocorrelation function for a suspension of noninteracting particles is given by (18):

$$(g(nT) - 1)^{1/2} = \exp(-nT/t_c)$$

T is the sample time and n is the channel number of the digital correlator. A polydisperse system yields a sum of exponential terms.

The correlation time t_c is related to the diffusion coefficient of the molecule by:

$$t_c = 1/k^2 D$$

k is the scattering vector.

Results

It has been shown that IF-3, even at concentrations as low as 1 mg/ml, may form aggregates in solution (Giri and Pon, submitted for publication). For this reason, the addition of IF-3 to the 30S ribosomal particles may also result in the transient aggregation of the subunits. To avoid this phenomenon, in the present hydrodynamic studies, we have compared 30S ribosomal subunits and 30S·IF-3 complexes isolated from sucrose gradients. This resulted in a greater homogeneity in the ribosomal samples and minimized undesirable aggregation.

Table I presents a summary of the hydrodynamic parameters determined for 30S ribosomes and 30S·IF-3 complex. It can be seen from the table that regardless of whether the ribosomes were heat-reactivated or not at 50°C for 5 min prior to the physical measurements there are virtually no differences in the sedimentation coefficient, intrinsic viscosity, and partial specific volume between 30S and 30S·IF-3 complex. Similarly, regardless of the presence of IF-3, no differences were found between ribosomes subjected or not subjected to heat-reactivation except for a difference in the intrinsic viscosity which increases 20-25% after heat reactivation.

These results are in disagreement with the previously published data (1) which indicated a large change in all the above-mentioned hydrodynamic parameters of the 30S ribosomal subunit upon IF-3 binding. It was decided, there-

Table I Physical properties of 30S subunits and 30S·IF-3 complex

Parameter	30S subunits	30S·IF-3complex
$s_{20,w}$ (S)	31.1 (31.0) ± 1.0	32.4 (31.9) ± 1.0
[η] (ml/g)	8.3 (6.2) ± 0.3	8.2 (6.5) ± 0.4
∅' (ml/g)	0.576 (0.580) ± 0.005	0.579 (0.577) ± 0.005

The values in parentheses refer to measurements performed without prior heat reactivation (50°C for 5 min).

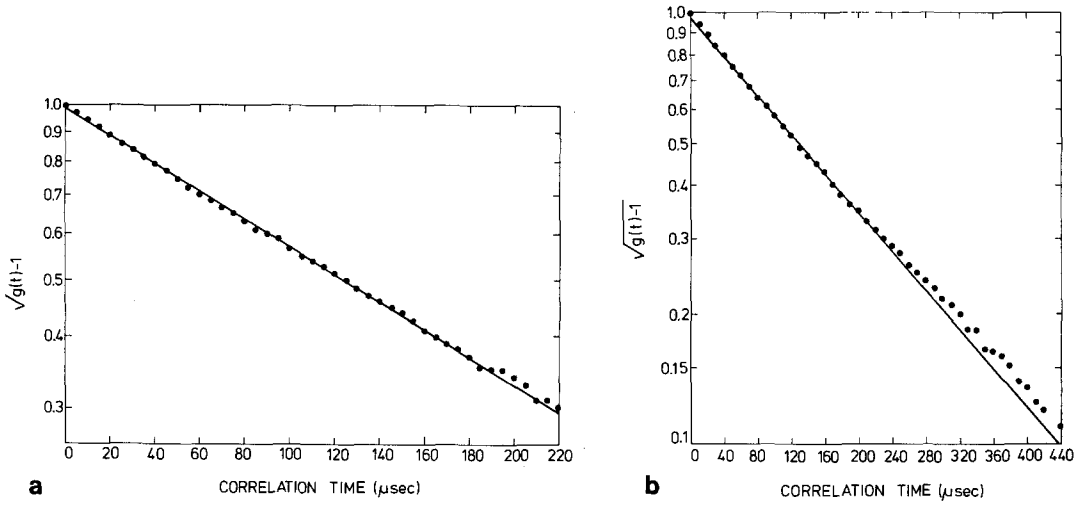

Fig. 1. Photon correlation. Autocorrelation function of 30S subunit on a 5 μsec (1A) and 10 μsec (1B) time scale. Theoretical curve (——) and experimental values (●).

fore, to supplement these data with laser light scattering measurements.

These measurements are particularly suitable to reveal overall differences in the size and shape of the ribosomal particles, but, unfortunately, are especially sensitive to interference from both aggregation and dust particles. To avoid the latter problem, buffers and ribosomal solutions were centrifuged at 10k rpm and filtered through a combination of Millipore filter membranes (0.22-0.60 μm). However, since it was found that 30S·IF-3 complexes do not withstand the filtration procedure, pre-formed complexes could not be used in these experiments. Instead, the measurements had to be performed after titrating 30S ribosomal subunits with increasing amounts of IF-3. To minimize possible aggregations, IF-3 was added from a very concentrated solution (8 mg/ml) in buffer (Tris-HCl, pH 7.7, 20 mM; NH_4Cl, 200 mM; EDTA, 0.1 mM; glycerol, 10%) containing 6 M urea. Control 30S ribosomal subunits received the same volumes of the above buffer without IF-3. The final concentration of urea in the samples never exceeded 200 mM.

In spite of these precautions, the 30S ribosomal subunit solution was not completely monodisperse as evident from the fact that, while on a 5 μsec time scale the correlation function deviates little from a single exponential function (Fig. 1A), on a 10 μsec time scale some deviation from linearity is observed (Fig. 1B). An additional complication arises from the fact that the distribution of the 30S aggregates tends to change with time following the dilutions of the 30S made prior to the measurements.

Table II Laser light scattering of 30S ribosomal subunits with or without IF-3

Sample		correlation times (microsec) time scale 5 μsec	time scale 10 μsec
1. 30S	after 30 min	185.0	-
	after 24 hrs	181.9	-
2. 30S+IF-3 in 6M urea buffer	after 30 min	184.4	189.8
	after 24 hrs	179.5	-
3. 30S+6M urea buffer	after 30 min	184.5	189.0
	after 24 hrs	181.0	-
4. 30S diluted (1:5)	after 30 min	-	-
	after 24 hrs	181.8	-

The experiment was performed at 20°C; scattering angle was 90°.

This is seemingly due to the establishment of new aggregation equilibria upon dilution. Therefore, to determine accurately the correlation time for the 30S ribosomal subunits with or without IF-3, several measurements had to be made at various times following dilution and addition of IF-3 (see Table II). It can be seen in the table that the correlation times of the 30S ribosomal subunits with or without IF-3 do not differ within the error of the measurement (2%). Since the correlation time is proportional to the average Stokes radius of the molecule, the change in this parameter upon addition of IF-3 should be smaller than 2%.

Discussion

The occurrence of a ribosomal conformational change has been postulated as the most plausible explanation for the properties acquired by the 30S ribosomes upon IF-3 binding (2), and its occurrence has since been substantiated by many lines of evidence (3-7), although its extent remains unknown.

The results of the present paper show, contrary to that previously reported (1), that the binding of IF-3 to the 30S ribosomal subunits does not result in a detectable change of the hydrodynamic properties of the ribosomal subunits. In addition to the standard hydrodynamic methods (see Table I), 30S and 30S·IF-3 complexes were compared by quasi-elastic light scattering. This method provides a precise and perturbation free determination of macromolecular diffusion coefficients. These are obtained by measuring the intensity autocorrelation function and correlation time of photons scattered by a suspension of diffusing particles. The accuracy of 2% that

can be achieved allows the use of this method as a sensitive tool for measurement of size and shape (but not of shape alone) changes of macromolecules. The results obtained (see Table II) showed no difference (within 2%) in the autocorrelation times of 30S and 30S·IF-3 complexes.

The lack of differences in the hydrodynamic parameters and in the autocorrelation times between 30S and 30S·IF-3 complexes does not prove that no conformational change takes place, but simply that the change in question is beyond the limits of detection of the methods employed. Indeed, the resolving power of the hydrodynamic methods and of the quasi-elastic light scattering is not adequate to resolve small conformational changes or, as mentioned above, even relatively large shape changes not accompanied by size changes.

Compared to the previous study (1), some improvements have been introduced in the present work, the main one consisting in the use, whenever possible, of 30S·IF-3 complexes and 30S ribosomal subunits isolated from sucrose gradients in order to avoid aggregation phenomena and to work with a more homogenous population of ribosomes. This procedure, however, has other drawbacks. Since saturation of the ribosomes with IF-3 is difficult to reach, the amount of IF-3 present in the 30S·IF-3 complexes analyzed never exceeded 0.7 copies of IF-3 per 30S particle, so that the maximum difference observable between 30S and 30S·IF-3 complexes would represent at most only 70% of the total effect. In spite of this limitation, we feel confident that no change in the hydrodynamic parameters occurs upon IF-3 binding within the limits of the experimental error of the methods employed. Therefore, although the previous results (1) may have been affected to some extent by aggregation phenomena, these cannot account quantitatively for the reported large change in hydrodynamic properties of the 30S ribosomes upon IF-3 binding for which we can provide no explanation.

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