CONFORMATIONAL TRANSITION OF THE 30 S RIBOSOMAL SUBUNIT INDUCED BY INITIATION FACTOR 3 (IF-3)

Hasko H. Paradies, Alfred Franz, Cynthia L. Pon and Claudio Gualerzi

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

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Summary: In this paper some physical-chemical properties of 30 S ribosomal subunits and 30 S - IF-3 complexes are reported. Evidence is presented that both sedimentation coefficient ane low-angle X-ray diffraction pattern of the 30 S ribosomal subunits are modified by the addition of IF-3, indicating that IF-3 induces a conformational change (operationally definable as a "tightening") of the ribosomal particle. The possible molecular mechanisms of the IF-3 - induced conformational transition of the ribosomal particle are discussed in relation to the properties of IF-3. It is proposed that a conformational change of the 30 S particles is the cause of the previously reported IF-3 - induced release of aminoacyl-tRNAs from the 30 S particles (1) as well as the basis for other IF-3 activities.

We have previously reported that initiation factor 3 (IF-3) is endowed with the unique property of releasing aminoacyl-tRNAs (with the only exception of the initiator fMet-tRNA) from their complexes with 30 S ribosomal subunits and synthetic polynucleotides (1). This property has since been used in our laboratory to assay for IF-3 activity during the various steps of its purification. The specific advantage of this method is that it involves a simple and rapid reaction which does not depend on the presence of the other two initiation factors (IF-1 and IF-2). The biological significance and the molecular mechanism of the IF-3 - mediated aminoacyl-tRNA release, however, have so far remained obscure. Also unclear has remained the relationship between this activity and the other properties displayed by IF-3 (2-4).

In the present paper, in an attempt to elucidate the molecular basis for IF-3 - directed release of aminoacyl-tRNA and for other activities promoted by IF-3, we have studied the effect of the addition of purified IF-3 on the conformation of isolated 30 S ribosomal subunits.

MATERIALS AND METHODS

For the preparation of 30 S ribosomal subunits, the entire procedure was carried out at 2-4°C unless otherwise specified. The S-30 fraction (5) obtained from 20-40 g of Escherichia coli MRE 600 (mid-log) cells was centrifuged for 3 1/2 h at 50,000 rpm in a Spinco 60 Ti rotor. The resulting pellets were carefully rinsed with a small volume of buffer (10 mM Tris-HCl,

pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 6 mM 2-mercaptoethanol) and washed by resuspending in buffer consisting of 100 mM Tris-HCl, pH 7.7, 10 mM Mg acetate, 50 mM KCl, 500 mM NH_4Cl and 6 mM 2-mercaptoethanol (1 ml/g of cells). After centrifugation at 15,000 rpm for 10 min, the supernatant fluid was centrifuged for 3 1/2 h at 50,000 rpm in a Spinco 60 Ti rotor. The entire washing procedure was repeated two more times. The final ribosomal pellet was resuspended in buffer containing 10 mM Tris-HCl, pH 7.7, 60 mM NH_4Cl, 6 mM 2-mercaptoethanol and 0.3 mM Mg acetate to a final concentration of approximately 25 mg/ml and dialyzed overnight at 2-4°C against 200 volumes of the same buffer. The ribosomal suspension was then incubated for 5 min at 50°C. The Mg^{2+} concentration was adjusted to 1 mM and ribosomes (500 - 600 mg) were quickly cooled and subjected to zonal centrifugation in a Spinco BXV rotor on a 7 - 38% sucrose density gradient (6) in 10 mM potassium phosphate buffer, pH 7.7, 60 mM NH_4Cl, 1 mM Mg acetate and 6 mM 2-mercaptoethanol at 25,000 rpm for 17 h. The 30 S subunits were collected from the gradient fractions by centrifugation for 12-15 h at 50,000 rpm in the Spinco 60 Ti rotor. Initiation factor IF-3 was purified as previously described (1) with the only exception being the omission of the heating step.

The 30 S - IF-3 complex was usually prepared by mixing 1.1 A_{260} units of 30 S ribosomal subunits with 16.5 μg (730 pmoles) of purified IF-3 in buffer containing 10 mM Tris-HCl, pH 7.8, 60 mM NH_4Cl, 6 mM 2-mercaptoethanol and 1 mM Mg acetate. Previous stoichiometric determinations had shown that at this IF-3:ribosome ratio all 30 S particles have one molecule of IF-3 bound. In some cases, however, a higher ribosome input was used as specified in Results.

Viscosities were measured in an Ostwald viscometer at 4°C with flow times of 143 sec for water. No corrections for adhesion or kinetic energies were made.

Ultracentrifugation of 30 S subunits and 30 S - IF-3 complexes, dissolved in 10 mM Tris-HCl, pH 7.8, containing 1 mM Mg acetate, 60 mM NH_4Cl was carried out in a Beckman Model E ultracentrifuge equipped with a photoelectric scanner and RTC-temperature control. Runs were usually performed at 15°C (unless otherwise specified) in a double sector cell with quartz windows at 30,000 rpm or at 40,000 rpm in an AN-F titanium rotor. The boundary of the sedimenting particles were followed by either schlieren optics or by UV scanner. In case of measurements with the photoelectric scanner at 260 nm, corrections of the $S^{\circ}_{20,w}$ value only for viscosity and density of the buffer, but not for the concentration of 30 S or 30 S - IF-3 complex. All $S^{\circ}_{20,w}$ values determined are reduced to standard conditions.

Low-angle X-ray scattering was performed as described under Fig. 1.

Table 1. Molecular properties of 30 S - IF-3 complex and the 30 S particle of E. coli MRE 600

	Parameters	30 S - IF-3 complex	30 S
M[a]	$S^{\circ}_{20,w}$ (sedimentation coefficient)	37.6±0.4	30.8±0.5
M	$D^{\circ}_{20,w}$ (diffusion coefficient)[c]	3.74±1.5 x 10^{7} cm^2/sec	2.95±0.5 x 10^{7} cm^2/sec
C[b]	f/f_o (actual frictional ratio)	1.032	1.182
C	p=b/a (shape, axial ratio)	1:1.8:1.8	1:4:4
M	$[\eta]_{c=o}$ (intrinsic viscosity)	6.2 ml/g	7.8 ml/g
M	$\bar{R}_g$ (radius of gyration)[d]	61.0±2.0 Å	67.8±1.5 Å
C	β[e]	2.13 x 10^{-6}	2.20 x 10^{-6}
C	Dimensions	92.5 x 138.5 x 138.5 Å	60 x 220 x 220 Å
M	Volume	1.25 x 10^{6} Å^3	1.40 x 10^{6} Å^3
M	Molecular weight[f]	0.87 x 10^{6}	0.85 x 10^{6}

[a] M = measured value; [b] C = calculated value; [c] Measured according to Paradies and Franz, (Biochemistry (1974) in press).
[d] The average radii of gyration from 15 measurements at 15°C have been listed. The lowest value obtained for the 30 S - IF-3 complex was 57.5 Å, the highest 62.8 Å, slightly depending on the preparation of IF-3.
[e] $\beta = [N/16.260\pi^2]^{1/3} (f_o/f)\vartheta^{1/3}$, according to Scheraga and Mandelkern (7).
[f] Measured by low - angle X-ray scattering

RESULTS:

A series of 25 sedimentation velocity measurements at different dilutions of either 30 S ribosomal subunits or complexes of 30 S and IF-3 were performed. These sedimentation studies yielded values (at infinite dilution) of 30.8 S for the 30 S particle and 37.6 S for the complex. In the presence

of a saturating amount of IF-3 (see Materials and Methods), only one peak of 37.6 S corresponding to the 30 S - IF-3 complex was always seen. Upon addition of more 30 S subunits, however, a second slower peak corresponding in $S^{o}_{20,w}$ value to 30 S subunits alone became increasingly apparent along with a peak of 30 S dimers sedimenting faster than the 30 S - IF-3 complex. The dimerization of the 30 S was seen to be concentration dependent and strongly reduced in the presence of IF-3. The temperature at which the sedimentation analysis was performed proved to be important, since at temperatures higher than 15°C (i.e. 20°C), the 30 S - IF-3 complex starts to dissociate. We have taken advantage of this fact by running the 30 S - IF-3 complex first at low temperature, then at high temperature, and again at low temperature to show a transition of the $S^{o}_{20,w}$ value from 37.6 S to 30.8 S and back to 37.6 S.

When the intrinsic viscosities of the 30 S particles and of the 30 S - IF-3 complex were measured, values of 7.8 ml/g and 6.2 ml/g were obtained. These values along with other hydrodynamic properties of the 30 S and the 30 S -IF-3 complex are listed in Table 1. If it is assumed that the apparent partial specific volume of the 30 S - IF-3 complex is still the same as that of the 30 S particle (0.605), it is clear from the table that the increase in the $S^{o}_{20,w}$ value of the 30 S particle following the binding of purified IF-3 is due to a change in the shape of the elliposoid of revolution of the 30 S particle with an axial ratio of 1:4:4 to a more spherical conformation with an axial ratio of 1:1.8:1.8.

The change in the conformation of the 30 S particle following the binding of IF-3 was also studied by measuring the scattered intensities of low-angle X-rays for solutions containing different concentrations of 30 S or 30 S - IF-3 complexes (Fig. 1). The values of the apparent radii of gyration (R_g) were plotted against concentration and then linearly extrapolated to infinite dilution. The average radii of gyration for 15 measurements are also listed in Table 1. The 30 S - IF-3 complexes were found to be sensitive to long exposures to X-rays and tended to dissociate. Nevertheless, the decrease of the R_g of the 30 S - IF-3 complex compared to the 30 S alone has been repeated several times at different concentrations of 30 S and IF-3.

In order to interpret the angular dependence of the scattering intensity of the 30 S and the 30 S - IF-3 complex, the theoretical scattering curves for 3 oblate ellipsoids of revolution having axial ratios of 1:4:4, 1:2.5:2.5 and 1:1.5:1.5 were calculated and plotted in Fig. 3 along with the particle scattering curves experimentally measured for the 30 S and the 30 S - IF-3 complex. As seen in the figure, the experimental curve for the 30 S particle

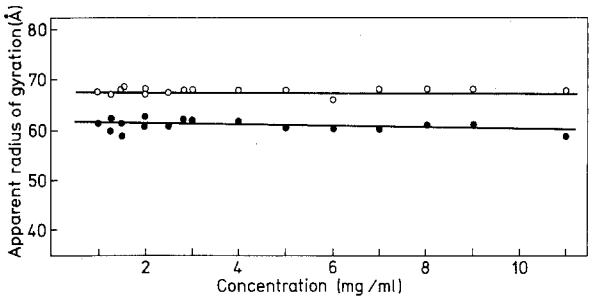

Figure 1. Determination of the apparent radii of gyration at infinite dilution for the 30 S and the complex. Solutions of 30 S and 30 S - IF-3 complex were measured in a Kratky camera, equipped with an electronically programmed step scanning device (Müller-Seifert), using CuK_{α}-radiation. It was observed that the complex of 30 S and IF-3 was very sensitive to long exposures to X-rays when the temperature was raised to 20°C. Scattering curves were recorded in specially designed capillary tubes at 15°C for dilute solutions containing different concentrations of 30 S and 30 S - IF-3 complex. The scattering of the buffer solution was subjected from each scattering curve. Sampleholders were 1 mm thick with 25 μ thick quartz windows. Slit sets were 0.15 and 0.20 mm for the 30 S particles and complex. The apparent radius of gyration was determined by plotting the logarithm of the scattered intensities ($\log I/I_o$) against $(2\theta)^2$ in the 0.9 I_o to 0.6 I_o region, where one normally obtains a fairly straight line and the slope of this line is proportional to the apparent R_g^2. The values of the apparent R_g were plotted against concentration and then linearly extrapolated to infinite dilution. •———• 30 S - IF-3 complex, ○———○ 30 S

is nearly coincident with the theoretical curve calculated for an oblate ellipsoid with an axial ratio of 1:4:4. The experimental curve for the 30 S - IF-3 complex, on the other hand, is clearly different from that of the 30 S and found to be intermediate between the theoretical curves calculated for more spherical particles having axial ratios of 1:2.5:2.5 and 1:1.5:1.5 and nearly coincident with the latter.

DISCUSSION:

In this paper the molecular properties of the 30 S ribosomal subunits have been compared with those of the 30 S - IF-3 complex. Several parameters of the 30 S particle, namely, sedimentation coefficient, radius of gyration, hydrodynamic volume, etc., were found to be significantly modified by the addition of IF-3. As mentioned above the 30 S - IF-3 complex was found to be heat-labile and sensitive to long exposure to X-rays. It was possible to see, therefore, that when the 30 S - IF-3 complex was dissociated as a result of an increase in temperature or of the long exposure to X-rays, the radius of gyration as well as the $S^{\circ}_{20,w}$ value of the original 30 S particles could be restored. Although one has to be careful in interpreting the change in the

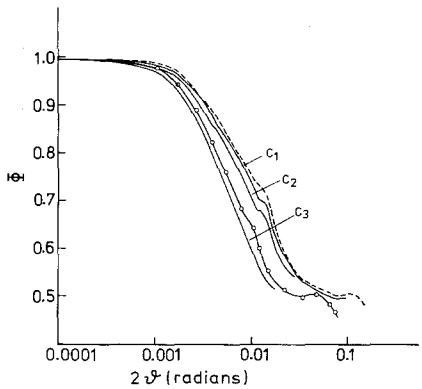

Figure 2. Experimental and theoretical scattering curves for 30 S and 30 S - IF-3 complex. The angular dependence of the scattering intensity $\Phi(2\vartheta)$ for various axial ratios (C_1=1:4:4; C_2=1:2.5:2.5; C_3=1:1.5:1.5) are compared with the experimental curves for the 30 S (- - - -) and 30 S - IF-3 complex (——— — .). These results are plotted in the normalized form $\Phi(\vartheta)=I(\vartheta)/I_o$ so that $\Phi(0)=1$.

axial ratio for the oblate ellipsoids because of possible salt and hydration effects on the partial specific volume of the ribosomal particles, taken together, these results strongly support the premise that the 30 S ribosomal subunit undergoes a modification of its shape from an ellipsoidal form to a more spherical form, becomes more compact and sediments faster when IF-3 is bound to it (see Fig. 3). IF-3 is a basic protein which binds specifically to the 30 S ribosomal subunit and whose primary binding target was postulated to be a segment of the 16 S rRNA (8). In other cases where a conformational transition of the ribosomes has been investigated by circular dichroism (9,10) a direct involvement of the rRNA has been implicated. In addition, unpublished observations (Yuki, Pon and Gualerzi) seem to indicate that IF-3 is capable of inducing a conformational transition of the "naked" (deproteinized) 16 S rRNA. It is possible, therefore, that the IF-3 - induced conformational change of the 30 S ribosomal subunits is due to a corresponding change of the rRNA conformation.

An additional explanation for the molecular mechanism of the conformational transition induced by IF-3 could be that the binding to the 30 S subunit of a highly positively charged molecule like IF-3 results in a local change of the net charge causing the repulsion of the positively charged ribosomal proteins or basic amino acid residues present near the binding site of IF-3. This, in turn, would result in a general stereochemical rearrangement of the macromolecular components of the 30 S particle.

Conformational changes of both ribosomal subunits have been reported to

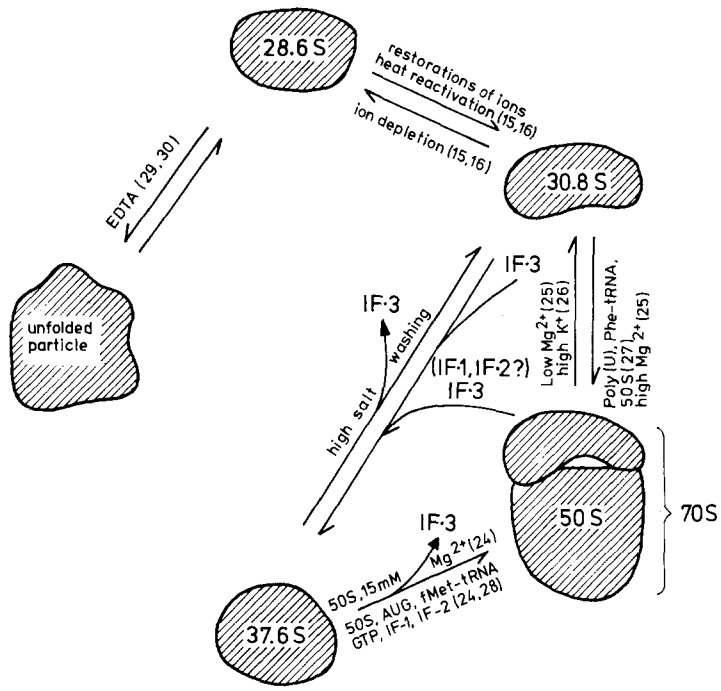

Figure 3. Scheme of the conformational transitions known to occur for the 30 S particle.

occur during the association (dissociation) process (10-14) and as a result of variations of the ionic environment (15-17) and functional activity (18, 19). A conformational transition resulting from the interaction of the 30 S particle with the initiation factors has also been postulated (20). A diagram summarizing the known conformational changes of the 30 S ribosomal subunit is presented in Fig. 3.

It is likely that the binding and release of different macromolecules to the 30 S ribosomal subunit is modulated by discrete changes of the particle conformation. The subunit anti-association activity of IF-3 (21,22), the absolute requirement for IF-3 in physiological binding of mRNA (23,24), the stimulation by IF-3 of amino acid incorporation directed by polynucleotides under suboptimal conditions (low polynucleotide concentration (23)) and the release of Phe-tRNA from poly U - 30 S complexes (1) are likely to be different manifestations of the same phenomenon, namely, a conformational transition of the 30 S particle triggered by the factor as described in this paper.

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