

## X-Ray and Neutron Small-Angle Scattering Studies of the Complex between Protein S1 and the 30-S Ribosomal Subunit

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X-ray and neutron solution scattering experiments have been done to investigate the influence of the binding of ribosomal protein S1 on the conformation of the 30-S ribosomal subunit of *Escherichia coli*. The following conclusions were made.

1. The alterations (if any) in conformation of the non-S1 parts of the 30-S subunit induced by S1 binding are too small to be detected ( $< 0.1$  nm change in radius of gyration).
2. The center of gravity of protein S1 bound to the 30-S subunit is quite far from the center of gravity of the particle ( $\approx 7.5$  nm).

Ribosomal protein S1 plays a key role in the attachment of mRNA to the 30-S ribosomal subunit [1, 2] as well as in initiation of Q $\beta$  RNA transcription [3]. Little is known, however, about the location of S1 and the nature of its interaction with either Q $\beta$  replicase or the 30-S subunit.

The fact that both the sedimentation constant and the electrophoretic mobility of 30-S subunits can decrease when protein S1 binds [4–6, 1] can be interpreted as evidence for the idea [7, 5] that S1 binding appreciably alters the conformation of the 30-S subunit. In support of this hypothesis is a recent report that S1 binding to the 30-S ribosome increases the susceptibility of three 30-S proteins to lactoperoxidase-catalyzed iodination (B. H. Sells, personal communication), clearly suggesting a conformational change in the 30-S subunit, the magnitude of which cannot, however, be easily assessed.

In order to understand the mechanism of action of protein S1 we thought it useful to quantify the impact of S1 binding upon the dimensions of the 30-S subunit using X-ray and neutron scattering techniques.

In this work, we concentrated mainly on radius of gyration analyses and, therefore, made extensive use of the parallel axes theorem [9, 10] to separate effects due to conformational changes from effects due to the simple physical presence of protein S1. This theorem can be stated as follows: assume an

aggregate exists consisting of one 30-S subunit depleted of protein S1 (desS1-30-S subunit or  $\beta$ ) and one S1 molecule ( $\alpha$ ). Then the radius of gyration of the aggregate ( $R_{\alpha\beta}$ ) can be expressed as a function of the radii of gyration of  $\alpha$  and  $\beta$  in the aggregate ( $R_\alpha$  and  $R_\beta$ ) and the distance between the centers of mass of  $\alpha$  and  $\beta$ ,  $\Delta_{\alpha\beta}$ :

$$R_{\alpha\beta}^2 = f_\alpha R_\alpha^2 + f_\beta R_\beta^2 + f_\alpha f_\beta \Delta_{\alpha\beta}^2$$

where  $f_\alpha$  and  $f_\beta$  are the scattering weights of  $\alpha$  and  $\beta$  relative to the scattering weight of the aggregate (weights are a function of the solvent). It is important to note that even in the absence of any conformational change upon binding,  $R_{\alpha\beta}$  can be larger or smaller than  $R_\beta$ . For example, if the location of the center of gravity of protein S1 and the center of scattering mass of the 30-S subunit were identical,  $R_{\alpha\beta}$  could be found to be as much as 0.38 nm smaller than the radius of gyration of the desS1-30-S subunit, when a neutron scattering experiment is performed in  $^2\text{H}_2\text{O}$  solvent. This decrease simply reflects the fact that  $R_\alpha$  is less than  $R_\beta$ .

X-ray scattering studies (in which contributions from RNA prevail) indicate that if binding of protein S1 induces a conformational change in the 30-S subunit, it is too small to be detected. On the other hand, small-angle neutron scattering, under conditions in which contributions from proteins predominate, point to a large increase in the radius of gyration of the 30-S subunit upon binding of S1. By contrast-matching S1 to the solvent, we show that this increase in radius of gyration must reflect a location for S1 far away

*Abbreviations.* desS1-30-S subunits, 30-S subunits lacking protein S1; desS1-30-S · S1, complex between desS1-30-S subunits and protein S1; desS1-30-S · [ $^2\text{H}$ ]S1, complex between desS1-30-S subunits and deuterated protein S1.

from the center of mass of the 30-S particle and not a conformational change induced in the 30-S subunit.

## MATERIALS AND METHODS

Ribosomes and proteins were isolated from *Escherichia coli* MRE600. *E. coli* grown in an 80.5%  $^2\text{H}_2\text{O}$  medium [11] was the source of deuterated protein S1, [ $^2\text{H}$ ]S1. After extraction from deuterated 70-S ribosomes by dialysis in 1 mM Tris (pH 7.5), the impure S1 was chromatographed on DEAE-cellulose [12] and further purified by Sephadex G-150 chromatography [13]. Dodecylsulfate gel electrophoresis revealed deuterated and normal S1 to be 92% and more than 97% pure, respectively. The deuterated S1 is expected to have about 61% of its nonexchangeable hydrogens replaced by deuterium [11,14]. desS1-30-S subunits were prepared by two methods: the Tris method [12], involving dialysis against 1 mM Tris, and the  $\text{NH}_4\text{Cl}$  method [2], involving the pelleting of 30-S subunits in 0.85 M  $\text{NH}_4\text{Cl}$ .

Samples were prepared as follows for X-ray and neutron scattering experiments. desS1-30-S subunits were incubated alone or with S1 for 10 min at 37 °C in 14 mM Tris, 7 mM magnesium acetate, 70 mM  $\text{NH}_4\text{Cl}$ , 0.4 mM EDTA, 6 mM 2-mercaptoethanol, pH 7.5. Pelleting followed over a 13-ml 6% sucrose cushion in 20 mM Tris, 10 mM magnesium acetate, 100 mM  $\text{NH}_4\text{Cl}$ , 0.5 mM EDTA, 6 mM 2-mercaptoethanol, pH 7.5 (Ti60 rotor, 35000 rev./min, 17 h). The pellets were then dissolved in buffer I (10 mM Tris, 50 mM KCl, 0.5 mM  $\text{MgCl}_2$ , 6 mM 2-mercaptoethanol, pH 7.5) and extensively dialyzed in the same buffer. The solvent in buffer I was pure  $\text{H}_2\text{O}$  for X-ray scattering experiments and pure  $^2\text{H}_2\text{O}$  for neutron scattering experiments. Control experiments showed that the binding properties of protein S1 to the 30-S subunit are independent of the deuterium content of either buffer I or S1 itself. The quantification of S1 bound to the pelleted samples was routinely checked by dodecylsulfate gel electrophoresis and agarose/acrylamide gel electrophoresis [13]. Samples were analyzed by agarose/acrylamide gel electrophoresis and by model E ultracentrifugation, using Schlieren optics and sample concentrations in the range employed for scattering experiments, both before and after data collection. No alterations were observed. The samples were free of 50-S subunit contamination and showed no sign of aggregation. The desS1-30-S samples were 98% free of S1 and the desS1-30-S · S1 samples were more than 90% free of desS1-30-S particles. The sedimentation coefficients ( $s_{20,w}$ ) of desS1-30-S and desS1-30-S · S1 preparations were  $30.0 \pm 0.3$  S and 29.9 S respectively (average of six measurements in buffer I).

Small-angle X-ray measurements were performed at 4 °C, using a Kratky camera, as previously described

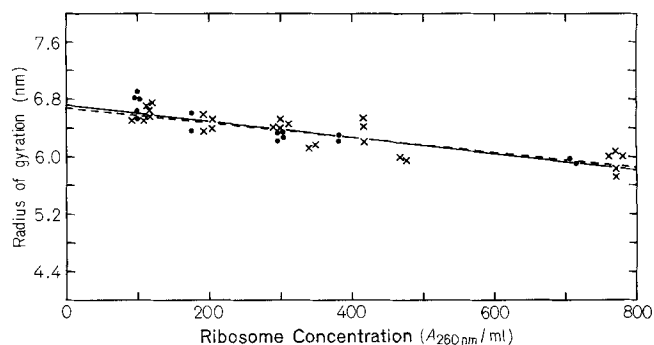


Fig. 1. X-ray diffraction results from 30-S subunits lacking protein S1 ( $\times$ , ----) and 30-S subunits saturated with S1 ( $\bullet$ , —). Scattered intensity was measured as a function of angle and a radius of gyration was extracted from the data (see Fig. 2). The concentration dependence of the radii of gyration measurements is as follows:  $(-1.13 \pm 0.23) \times 10^{-3} \text{ nm} \cdot A_{260}^{-1} \cdot \text{ml}$  for the desS1-30-S · S1 samples and  $(-1.02 \pm 0.14) \times 10^{-3} \text{ nm} \cdot A_{260}^{-1} \cdot \text{ml}$  for the desS1-30-S samples. The observed radii of gyration at infinite dilution are  $6.69 \pm 0.035$  nm for the desS1-30-S samples and  $6.72 \pm 0.04$  nm for the desS1-30-S · S1 samples. Errors are standard errors of the mean

[13]. Entrance and exit slits were respectively 100 and 200  $\mu\text{m}$  wide. Small-angle neutron scattering was performed using a Söller slit diffractometer as previously described [11].

## RESULTS

### X-Ray Radius of Gyration does not Depend on Binding of Protein S1

Fig. 1 presents the results of X-ray diffraction experiments. Plotted are the radii of gyration of 30-S subunits depleted of protein S1 or saturated with S1, as a function of concentration. It is apparent that the X-ray radius of gyration of the 30-S subunit is not dramatically affected by addition of S1. This result makes it unlikely that S1 binding induces a major conformational change in the 30-S subunit. Small RNA conformational changes are not ruled out, however, and large changes in the protein moiety of the 30-S subunit are possible, since the low-angle X-ray scattering from a 30-S subunit is dominated by RNA and is therefore relatively insensitive to changes in protein conformation. (The X-ray scattering density of RNA, relative to a water solvent, is 2.07 times larger than the scattering density of protein [10].)

### Neutron Radius of Gyration does Depend on Binding of Protein S1

In order to look more carefully at the protein distribution of the 30-S subunit, we turned to neutron scattering in a  $^2\text{H}_2\text{O}$  solvent. Protein has a neutron scattering density, relative to this solvent, 95% larger than RNA [15]. Fig. 2 shows typical Guinier plots

of low-angle data for 30-S subunits with and without protein S1. It is obvious here that the two preparations scatter differently. The slope of a Guinier plot is proportional to the square of the radius of gyration.

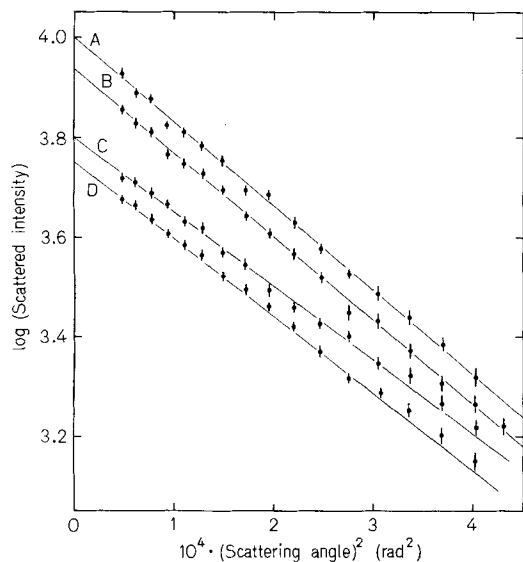


Fig. 2. Neutron scattering study of the effect of S1 binding on the radius of gyration of 30-S subunits. Shown are examples of Guinier plots [26] for (D) 30-S subunits lacking S1 (desS1-30-S), (A, B) desS1-30-S complexed with normal S1 (desS1-30-S · S1) and (C) desS1-30-S complexed with partially deuterated S1 (desS1-30-S · [<sup>2</sup>H]S1), which contains enough deuterium to match its scattering density with that of the solvent (100% <sup>2</sup>H<sub>2</sub>O). Each data set is fitted with a straight line by a variance-weighted least-squares procedure and the radius of gyration is obtained from the slope,  $\alpha$ , as  $R = 0.04183 \sqrt{-\alpha}$ . The data are arbitrarily scaled with respect to log (scattered intensity) for plotting convenience. The radii of gyration obtained in these particular examples are (A)  $7.24 \pm 0.05$  nm, (B)  $7.25 \pm 0.05$  nm, (C)  $6.86 \pm 0.06$  nm and (D)  $6.95 \pm 0.05$  nm. The one  $\sigma$  error of each measurement is represented by a bar of appropriate height. The neutron wavelength used for the experiments was 0.423 nm

We observe, therefore, that binding of S1 significantly increases the radius of gyration of 30-S subunits.

Table 1 summarizes our neutron scattering data. It appears that addition of S1 increased the radius of gyration of the 30-S subunit by  $0.325 \pm 0.09$  nm. Two alternative interpretations could be drawn from this S1-dependent increase in radius of gyration: (a) the center of gravity of S1 lies far away from the center of mass of the 30-S subunit; (b) S1 induces a conformational change in the ribosome. This latter change would have to occur predominantly in the protein moiety, however, because the X-ray radius of gyration is unchanged by S1 binding (Fig. 2).

*The Increase in Neutron Radius of Gyration is not due to a Conformational Change in the 30-S Subunit*

To discover which of the two previous interpretations correspond to reality, we prepared protein S1 deuterated in such a way that it has the same scattering density as the <sup>2</sup>H<sub>2</sub>O solvent (Materials and Methods). The binding properties of this S1 were identical to those of normal S1. The S1-dependent electrophoretic conversion from fast band to slow band [13] occurred as usual; both dodecylsulfate and agarose/acrylamide gel electrophoresis confirm that, after pelleting desS1-30-S-subunit · S1 complexes through a sucrose cushion (Materials and Methods), the S1 content of the complexes was independent of whether normal or deuterated S1 was used. Yet, neutron scattering from the complex of deuterated S1 and desS1-30-S subunits gave a radius of gyration identical to the radius of gyration of desS1-30-S particles (Fig. 2). This result proves that the increase of radius of gyration seen when normal S1 is used is due to the scattering contribution of S1 to the S1 · 30-S-subunit complex in accordance with possibility (a). It also implies that

Table 1. Effect of S1 binding on the radius of gyration of 30-S subunits

Extrapolation to infinite dilution of all observed radii of gyration was done using the concentration dependence observed from the X-ray scattering experiment. The concentration dependencies observed by either neutron or X-ray scattering were consistent with each other, but the X-ray concentration dependence curve is known with higher accuracy because more data points were collected. All errors indicated are standard errors of the mean.  $\Delta R_g = [R_g \text{ of desS1-30-S} \cdot \text{S1}] - [R_g \text{ of desS1-30-S}]$ . The X-ray scattering geometry (slit-shaped aperture) was different from the neutron scattering geometry (circular aperture), explaining the different slit smearing correction factors

Experimental technique	30-S subunit preparation	Corrected radius of gyration, $R_g$	Slit smearing correction	$\Delta R_g$	Number of experiments
		nm			
X-ray	NH <sub>4</sub> Cl desS1-30-S · S1	$7.00 \pm 0.04$	+ 0.28	$0.03 \pm 0.053$	15
	NH <sub>4</sub> Cl desS1-30-S	$6.97 \pm 0.035$	+ 0.28		26
Neutron	NH <sub>4</sub> Cl desS1-30-S · S1	$7.35 \pm 0.07$	+ 0.09	$0.31 \pm 0.10$	6
	NH <sub>4</sub> Cl desS1-30-S	$7.04 \pm 0.07$	+ 0.09		6
	NH <sub>4</sub> Cl desS1-30-S · [ <sup>2</sup> H]S1	$7.06 \pm 0.06$	+ 0.09		6
	Tris desS1-30-S · S1	$7.48 \pm 0.05$	+ 0.09		2
	Tris desS1-30-S	$7.11 \pm 0.07$	+ 0.09		2

S1 binding does not change the conformation of the desS1-30-S moiety to an appreciable extent or, less probably, that S1 binding results in shrinkage of part of the 30-S subunit and expansion of another, such that the radius of gyration of the whole desS1-30-S moiety is left unchanged. (Note in this regard that the high-angle scattering profiles from all our 30-S subunit preparations, whether desS1-30-S, desS1-30-S · S1 or desS1-30-S · [2H]S1, were similar and consistent with the subunits having an oblate spheroid shape of axial ratio 3–4:1). A comparative measurement of the forward scattering<sup>1</sup> ( $I_0$ ) per  $A_{260\text{ nm}}$  unit showed that the  $(I_0/A_{260\text{ nm}})^{1/2}$  of the desS1-30-S · [2H]S1 sample was 0.98–1.00  $(I_0/A_{260\text{ nm}})^{1/2}$  of the desS1-30-S sample, indicating good contrast matching of deuterated S1 by the solvent.

#### *Estimation of the Distance Separating Protein S1 from the Center of Mass of the 30-S Subunit*

By virtue of the parallel axes theorem it is possible to estimate roughly how far protein S1 must be from the center of mass of the 30-S subunit in order to obtain the increase in neutron radius of gyration we observe when using normal S1. The degree of information we can obtain from our data is however limited by the fact that 30-S subunits have two S1 binding sites [13].

We estimate that about 75% of the subunits in our desS1-30-S · S1 preparations had one S1 bound and 25% two S1 bound: gel electrophoresis in conditions which resolved 30-S subunits with one S1 bound from those with two S1 bound [13] revealed that 20–30% of the subunits in our desS1-30-S · S1 or desS1-30-S · [2H]S1 samples had two S1 bound; similarly, a comparative measurement of the forward scattering per  $A_{260\text{ nm}}$  unit indicated that normal S1 increases  $(I_0/A_{260\text{ nm}})^{1/2}$  by 15–17% (the binding of one mole of S1/mole of subunit would have caused only a 13% increase), in good agreement with the gel result.

The binding constants for S1 interaction with the two binding sites have been measured and, assuming the two sites are independent, it is found that the two site-binding constants [17] differ by a factor of about 35 [18]. In consequence, among the 30-S subunits with only one S1 bound, the proportion with the S1 bound to the weak-affinity site should be negligible. We therefore assume that the scattering from our desS1-30-S · S1 preparations is contributed, for all practical purpose, by a mixture of only two different samples: 30-S subunits with one S1 bound on the high-affinity binding site (75% of the total material) and 30-S subunits with two S1 bound (25% of the material). The value obtained for the radius of gyra-

tion of such a mixture is a weighted average of the individual radii of gyration [16]:

$$R_{\text{obs}}^2 = 0.71 R_1^2 + 0.29 R_2^2 \quad (1)$$

where  $R_{\text{obs}}$  is the observed radius of gyration of the desS1-30-S · S1 preparation,  $R_1$  and  $R_2$  are the radii of gyration of 30-S subunits with one and two S1 bound, respectively. Using the parallel axes theorem:

$$R_1^2 = 0.115 R_x^2 + 0.885 R_\beta^2 + 0.102 \Delta_1^2 \quad (2)$$

$$R_2^2 = f_{2\alpha} \left[ R_x^2 + \left( \frac{\vec{\Delta}_1 - \vec{\Delta}_2}{2} \right)^2 \right] + f_{2\beta} R_\beta^2 + f_{2\alpha} f_{2\beta} \left( \frac{\vec{\Delta}_1 + \vec{\Delta}_2}{2} \right)^2$$

$$R_2^2 = 0.206 R_x^2 + 0.794 R_\beta^2 + 0.0925 (\Delta_1^2 + \Delta_2^2) - 0.021 \Delta_1 \cdot \Delta_2 \quad (3)$$

where  $R_x$  and  $R_\beta$  are the respective radii of gyration of the S1 and the desS1-30-S moiety,  $f_\alpha$  and  $f_\beta$  are the scattering contributions of the S1 and the desS1-30-S moiety when the subunit has one S1 bound,  $f_{2\alpha}$  and  $f_{2\beta}$  are the equivalent scattering contributions when the subunit has two S1 bound,  $\vec{\Delta}_1$  and  $\vec{\Delta}_2$  are vectors from the center of scattering mass of the 30-S subunit to the center of gravity of S1, when bound to the high-affinity and the low-affinity binding site, respectively. The weighting factors  $f_\alpha, f_\beta, f_{2\alpha}, f_{2\beta}$  were computed as outlined in Table 2.

Expressions (2) and (3) can be substituted into Eqn (1) which then becomes after replacement of  $R_{\text{obs}}$  and  $R_\beta$  by their measured values:

$$11.07 \pm 2.01 \text{ nm}^2 = 0.1414 R_x^2 + 0.0991 \Delta_1^2 + 0.0268 \Delta_2^2 - 0.00617 \Delta_1 \cdot \Delta_2 \quad (4)$$

Clearly, information can be extracted from Eqn (4) only if upper limits are set for the values of  $R_x$ ,  $\Delta_1$  and  $\Delta_2$ . All studies so far published of the 30-S subunit overall dimensions predict that the longest linear dimension of the subunit is about 22.0 nm [19]. We therefore fix the maximum value of  $\Delta_1$  and  $\Delta_2$  at 11.0 nm. Similarly, the radius of gyration of S1 in solution is 5.8 nm [13], suggesting that S1 may be as long as 26.0 nm in solution: it seems reasonable to consider that the radius of gyration of S1, when bound to the 30-S subunit, is 5.8 nm or less. Table 3 presents the possible values of  $\Delta_1$  as a function of  $R_x$ ,  $\Delta_2$  and the cosine of the angle between  $\Delta_1$  and  $\Delta_2$ . Two conclusions emerge (which are consistent with the X-ray scattering measurements): (a)  $\Delta_1 = 7.8 \pm 3.2$  nm and (b) it is impossible for both  $\Delta_1$  and  $\Delta_2$  to be smaller than 5.3 nm.

<sup>1</sup> Forward scattering is the scattering at zero angle, experimentally obtained by extrapolation of the low-angle data; it is proportional to the square of the scattering weight [16].

Table 2. Neutron and X-ray scattering weights of substances used in this work

$f$  values are scattering weights relative to the scattering weight of the complex of one S1 with one desS1-30-S subunit. Protein refers to the protein moiety of a desS1-30-S subunit. Therefore:  $f_{S1} + f_{16-S RNA} + f_{protein} = 1$ , and  $f_{16-S RNA} + f_{protein} = f_{des1-30-S}$ . [ $^2H$ ]S1 refers to deuterated S1 (Materials and Methods). The scattering weights were computed using published values of scattering densities and partial specific volume for the RNA and protein moiety of the 30-S subunit [15], a molecular weight of 65000 for S1, 550000 for 16-S RNA and 298750 [11] for the protein moiety of a desS1-30-S subunit

Radiation	$f$ value for				$f_{16-S RNA}$ $f_{protein}$
	S1	[ $^2H$ ]S1	16-S RNA	protein	
Neutron in $^2H_2O$ solvent	0.115	0.008	0.355	0.53	0.67
X-ray	0.0554	0.0554	0.689	0.256	2.7

Table 3. The dependence of  $\Delta_1$  on the value of  $R_x$ ,  $\Delta_2$  and the cosine of the angle between  $\vec{\Delta}_1$  and  $\vec{\Delta}_2$ 

$\vec{\Delta}_1$  and  $\vec{\Delta}_2$  are the distances from the center of scattering mass of the 30-S subunit to the center of gravity of S1, when bound to the high-affinity and the low-affinity binding site respectively.  $R_x$  is the radius of gyration of S1 when bound to the 30-S subunit. If S1 is a sphere,  $R_x = 2.2$  nm; if it has the same structure as in solution,  $R_x = 5.8$  nm [13]

$R_x$	$\Delta_2$	$\frac{\vec{\Delta}_1 \cdot \vec{\Delta}_2}{\Delta_1 \Delta_2}$	$\Delta_1$
nm			nm
5.8	0		$8.4 \pm 0.8$
	11	+1	$6.4 \pm 1.1$
	11	-1	$5.7 \pm 1.1$
2.2	0		$10.4 \pm 0.6$
	11	+1	$9.2 \pm 0.8$
	11	-1	$8.5 \pm 0.8$

From the present data,  $\Delta_2$  and  $|\Delta_1 - \Delta_2|$ , i.e. the distance between the two S1 centers of mass, are indeterminate. Independent approaches could lead to the measurement of  $|\Delta_1 - \Delta_2|$  [20] and of  $R_x$  (our unpublished results). Should  $R_x$  and  $|\Delta_1 - \Delta_2|$  be measured, an estimation of  $\Delta_2$  would be possible and, obviously, a great reduction in the uncertainty of  $\Delta_1$  would result (see Table 3). However, even in the absence of this extra information, it seems clear that the center of mass of S1, when bound to the high-affinity binding site, is far from the center of the subunit.

## DISCUSSION

We would have liked to study the scattering behavior of 30-S subunit with protein S1 bound only on the high-affinity binding site in order to obtain a more accurate value for the distance between the

center of gravity of S1 and the center of mass of the 30-S subunit. The preparation of such samples, however, requires the subunits to be immersed in a high-magnesium buffer [13]. We found it unrewarding to scatter from 30-S subunits in a buffer containing more than 2 mM magnesium, because of 30-S subunit dimer formation. ( $^2H_2O$  increases the tendency of subunits to dimerize as noted by others [22].) We therefore resorted to a low-magnesium, but completely dimer-free, buffer for our experiments. This buffer, on the other hand, allows formation of a second, weak binding site for S1 [13].

Other groups have previously measured the radius of gyration of 30-S subunits without, however, attaching importance to the S1 content of their preparations. Our X-ray results, which are almost independent of S1 binding, agree very well with those of Hill et al. [23]. Our neutron results, in  $^2H_2O$  solvent, are more difficult to compare since they are sensitive to the amount of bound S1 per 30-S subunit. We obtain 7.06 nm for the radius of gyration of 30-S subunits depleted of S1 and 7.38 nm for 30-S subunits with 1.25 molecules S1 bound. Our results support those of Moore et al. [11] and suggest that the radii of gyration measured in  $^2H_2O$  by Beaudry et al. [22] might be slightly large (by a few tenths of a nanometer). Two related reasons might explain this discrepancy. (a) The scattering buffers used by Hill et al. [23], Moore et al. [11] and ourselves are very similar (10 mM Tris, 0.5–1 mM  $Mg^{2+}$ , 50–70 mM KCl) while the buffer used by Beaudry et al. contains 5–10 times more magnesium. (b) The high ratio of divalent to monovalent cations used by Beaudry et al. [22] leads to dimerisation of 30-S subunits as they noted. It is difficult to correct data for such an effect, which results in an overestimation of the radius of gyration of the monomer species [16].

Our scattering results show that S1 binding does not change the conformation of the 30-S subunit or that if a conformational alteration takes place, it leaves the radius of gyration of the desS1-30-S moiety unchanged. Independent of which of these two possibilities is correct, we also find that the center of gravity of S1, when bound to the high-affinity binding site, is more than 4.6 nm away from the center of mass of the 30-S subunit.

While it is true that radii of gyration are the most direct and least ambiguous data obtainable from small-angle experiments, it is also true that more than just the radius of gyration of a structure can be obtained from its solution scattering profile (e.g. molecular volume, molecular weight, etc. [16]). However, in view of the essentially negative outcome of the radius of gyration results reported above and the data interpretation problems inherent in dealing with mixtures of particles, as in the S1 complex populations, a more extensive analysis was not undertaken.

An analogy can be drawn between the interaction of S1 and IF-3, two functionally related proteins [2], with the 30-S subunit. Both proteins seem to induce some conformational change in the subunit [24, 25, and B. H. Sells, personal communication]. The neutron scattering studies of Beaudry et al. [22] with IF-3 and our studies with S1 indicate, however, that these conformational changes are likely to be local in nature and not to represent an upheaval of the 30-S ribosomal subunit structure.

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## REFERENCES

1. Szer, W. & Leffler, S. (1974) *Proc. Natl Acad. Sci. U.S.A.* **71**, 3611–3615.
2. Steitz, J. A., Wahba, A. J., Laughrea, M. & Moore, P. B. (1977) *Nucl. Acids Res.* **4**, 1–15.
3. Kamen, R., Kondo, M., Romer, W. & Weissmann, C. (1972) *Eur. J. Biochem.* **31**, 44–41.
4. Dahlberg, A. E. (1974) *J. Biol. Chem.* **249**, 7673–7678.
5. Littlechild, J. & Spencer, M. (1973) *Biochemistry*, **12**, 3102–3108.
6. Hermoso, J. M., Boublik, M. & Szer, W. (1976) *Arch. Biochem. Biophys.* **175**, 181–184.
7. Szer, W., Hermoso, J. M. & Leffler, S. (1975) *Proc. Natl Acad. Sci. U.S.A.* **72**, 2325–2329.
8. Reference deleted.
9. Kittel, C., Knight, W. D. & Ruderman, M. A. (1973) *Mechanics, Berkeley Physics Course*, vol. I, McGraw Hill, New York.
10. Engelman, D. M. & Moore, P. B. (1975) *Annu. Rev. Biophys. Bioeng.* **4**, 219–241.
11. Moore, P. B., Engelman, D. M. & Schoenborn, B. P. (1975) *J. Mol. Biol.* **91**, 101–120.
12. Tal, M., Aviram, M., Kamarek, A. & Weiss, A. (1972) *Biochim. Biophys. Acta*, **281**, 381–392.
13. Laughrea, M. & Moore, P. B. (1977) *J. Mol. Biol.* **112**, 399–421.
14. Moore, P. B. (1977) *Anal. Biochem.* **82**, 101–108.
15. Crichton, R. R., Engelman, D. M., Haas, J., Koch, M. H. J., Moore, P. B., Parfait, R. & Stuhmann, H. B. (1977) *Proc. Natl Acad. Sci. U.S.A.*, **74**, 5547–5550.
16. Beeman, W. W., Kaesberg, P., Anderegg, J. W. & Webb, M. B. (1957) in *Handbuch der Physik* (Flügge, S., ed.) vol. 32, pp. 321–442, Springer-Verlag, Berlin.
17. Klotz, I. M. (1973) *Acc. Chem. Res.* **7**, 169–169.
18. Laughrea, M. & Moore, P. B. (1968) *J. Mol. Biol.*, in the press.
19. Van Holde, K. E. & Hill, W. E. (1974) in *Ribosomes* (Nomura, M., Tissieres, A. & Lengyel, P., eds) pp. 53–91, Cold Spring Harbor Press, New York.
20. Moore, P. B. & Engelman, D. M. (1977) *J. Mol. Biol.* **112**, 228–234.
21. Reference deleted.
22. Beaudry, P., Petersen, H. U., Grunberg-Manago, M. & Jacrot, B. (1976) *Biochem. Biophys. Res. Commun.* **72**, 391–397.
23. Hill, W. E., Thompson, J. D. & Anderegg, J. W. (1969) *J. Mol. Biol.* **44**, 89–102.
24. Gualerzi, C., Grandolfo, M., Paradies, H. H. & Don, C. (1975) *J. Mol. Biol.* **95**, 569–573.
25. Michalski, C. J., Sells, B. H. & Wahba, A. J. (1976) *FEBS Lett.* **71**, 347–350.
26. Guinier, A. (1939) *Ann. Phys. (Paris)* **12**, 161–237.

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