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Molecular interactions between ribosomal proteins—An analysis of S7–S9, S7–S19, S9–S19 and S7–S9–S19 interactions*

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Abstract. Ribosomal proteins S7, S9 and S 19 from *Escherichia coli* have been studied by the sedimentation equilibrium technique for possible intermolecular interaction between pairs of proteins as well as in a mixture of 3 proteins. The proteins were isolated to a purity greater than 95% and were characterized in the reconstitution buffer. It was observed that none of the proteins has a tendency to self-associate in the concentration range studied in the temperature range 3–6°C. Protein S9 behaves differently in the presence of other proteins. Analysis of the sedimentation equilibrium data for S7–S9, S9–S19 and S7–S9–S19 complexes revealed the need for considering the presence of a component of higher molecular weight in the system along with the monomers and their complexes to provide a meaningful curve-fitting of the data. Proteins S7 and S19 were found to interact with an equilibrium constant of association of $3 \pm 2 \times 10^4$ M^{-1} at 3°C with a Gibbs free energy of interaction $\Delta G°$ of -5·7 kcal/mol. These data are useful for the consideration of the stabilization of the 30S subunit through protein-protein interactions and also help in building a topographical model of the proteins of the small subunit from an energetics point of view.

Keywords. Ribosomal proteins; S7, S9 and S19; interaction; equilibrium constant; 30S ribosome; free energy of interaction.

Introduction

The ribosome of *Escherichia coli* has been the centre of interest for a number of studies from both the functional and physical points of view. Several attempts have been made to understand the topography of the 30S subunit of the ribosome from the first assembly map of Mizushima and Nomura (1970) using various techniques (Hardesty and Kramer, 1985) such as chemical crosslinking (Bickle *et al.,* 1972; Chang and Flaks, 1972; Shih and Craven, 1973), chemical modification of the proteins (Craven and Gupta, 1970; Huang and Cantor, 1972), fluorescence energy transfer studies (Huang *et al.,* 1975), fragmentation studies (Miller and Sypherd, 1973; Morgan and Brimacombe, 1973; Schendel *et al.,* 1972), electron microscopy analysis of antibody-crosslinked structures (Tischendorf *et al.,* 1974; Lake and Kahan, 1975), neutron scattering studies (Engleman *et al.,* 1975; Langer *et al.,* 1978) and small-angle X-ray scattering studies (Osterberg *et al.,* 1976; Paradies and Franz 1976; Laughrea and Moore, 1977).

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Abbreviations used: TMK, Tris (0·03 M), $MgCl_2$ (0·02 M), KCl (0·35 M) pH 7·36 containing 2-mercaptoethanol; GuHCl, guanidine hydrochloride; 6 M urea buffer—0·05 M sodium phosphate, 0·012 M methylamine hydrochloride and 7×10^{-4} M 2-mercaptoethanol; pH 5·6; SDS, sodium dodecyl sulphate; PAGE, Polyacrylamide gel electrophoresis; M_r, molecular weight.

The direct physical interaction of protein pairs in the 30S ribosomal subunit has been studied by the method of sedimentation equilibrium using the analytical ultracentrifuge (Aune and Rohde, 1977; Aune, 1978; Prakash and Aune, 1978a, b, c). In the present approach, the proteins under consideration are isolated, characterized in solution, and mixed in known mass ratios and analysed by the technique of sedimentation equilibrium. The numerical techniques involved are discussed in the earlier references (Rohde *et al.,* 1975; Rohde and Aune, 1975; Aune and Rohde, 1977; Aune, 1977, 1978). The selection of the proteins has been narrowed down to those that have already been shown to be in proximity, behave as monomers in solution, are of functional importance and are obtainable pure in considerable quantity.

Proteins S7, S9 and S19 are of interest in the present study since S7 is one of the proteins of the 30S subunit that interact with 16S RNA (Zimmermann, 1974; Brimacombe, 1976; Garrett *et al.,* 1974) and S7, S9 and S19 have been shown to be close to each other and to the 3'-proximal region of 16S RNA (Yuki and Brimacombe, 1975; Zimmermann *et al.,* 1975; Rinke *et al.,* 1976). The proteins S7, S9 and S19 have been shown to be part of a ribonucleoprotein fragment from the 30S subunit (Morgan and Brimacombe, 1973). The proteins S9 and S19 have been shown to enhance the binding of S7 to 16S RNA (Nomura and Held, 1974). The protein S7 is also the rate-limiting component in the assembly of S6, S7, S8, S9 and S16 (Schlessinger, 1974). The proteins S7 and S9 have been crosslinked by several workers (Lutter *et al.,* 1972; Lutter and Kurland, 1973; Bode *et al.,* 1974; Lutter *et al.,* 1974, 1975; Sommer and Traut, 1975; Expert-Bezancon *et al.,* 1977; Langer *et al.,* 1978). Further, Bickle *et al* (1972) have crosslinked proteins S6, S7 and S9 using dimethyl suberimidate. Antibody to S19 inhibits Fmet-tRNA binding according to Traut *et al* (1974). In addition, Rummel and Noller (1973) have clearly shown that protein S19 is protected from trypsin digestion by the prior binding of tRNA to the ribosome.

These data aid in visualizing a stable topographical model of the proteins of the 30S subunit of *E. coli* from an energetics point of view. In this paper the isolation and characterization of proteins S7 and S19 and the interaction between the proteins S7 and S9, S7 and S19, and S9 and S19, and between the3 proteins in the ternary complex S7–S9–S19 are reported.

Materials and methods

Isolation of S7, S9 and S19

The 30S and 50S ribosomal subunits were isolated from *E. coli* MRE 600 cells as described by Rohde *et al* (1975). The individual fractions containing a fair amount of S7, S9 and S19 were obtained by chromatography on a phosphocellulose column using the procedure of Hardy *et al* (1969) and Rohde *et al.* (1975). The proteins S7, S9 and S19 were tentatively identified depending on their mobility in gel electrophoresis in the presence of urea and positions as described by Hardy *et al.* (1969) and Rohde *et al* (1975).

The fraction from the phosphocellulose column containing protein S7 along with protein S3 or S16, which coelute, was loaded onto a Sepharose 4B–100 column (1·5 × 85 cm) in 6 M guanidine hydrochloride (GuHCl) containing 0·001 M 2-mer-

captoethanol. The protein was further purified by gel filtration through a Sephacryl column (1·5 × 85 cm) in 6 M urea buffer, pH = 5·6. The protein S7, obtained pure after the above procedure, was pooled, dialysed free of salt, and lyophilized. The homogeneity of the sample was judged by gel electrophoresis in the presence of urea and by a modified sodium dodecyl sulphate (SDS) gel electrophoresis (Weber and Osborn, 1969; Rohde *et al.,* 1975). The gels stained with amido black were scanned in an ISCO UA-5 absorbance monitor at 546 nm.

The fraction containing primarily S19 also contained small quantities of S14, S15, and S12 or S13. The fraction was loaded on a Sephadex G-100 column (1·6 × 76 cm) in 6 M urea buffer, pH 5·6. Protein S19 elutes as the major peak with two other minor peaks eluting just after the void volume. The protein S19 obtained thus was reloaded on the Sephadex G-100 column and obtained in 95% homogeneity. The homogeneity of the protein was tested by the methods described for S7.

The isolation of protein S9 has been described earlier (Prakash and Aune, 1978c).

Amino acid analysis

Amino acid analysis of the proteins was carried out in a Beckman Model 121 amino acid analyser by the method of Spackman *et al* (1958). The amino acid analysis was compared with the published data of Craven *et al.* (1969) and Kaltschmidt *et al.* (1970) and the tentative assignments made earlier were confirmed by computing a correlation coefficient with the amino acid values available in the literature for the above proteins.

Absorptivity

Absorptivity of the proteins was determined in an analytical ultracentrifuge (Aune and Timasheff, 1971) using a synthetic boundary cell. Absorptivity at 280 nm of S7 and S19 was determined in 15% acetic acid at 20°C because the proteins were not of a sufficient concentration in Tris–$MgCl_2$–KCl (TMK) buffer for an accurate measurement. Lysozyme was used as the calibrating protein. Absorptivity was assumed to be the same in both TMK buffer and 15% acetic acid for the purpose of calculating the concentration of the proteins.

Refolding

The proteins were refolded in TMK buffer. The lyophilized proteins (0·5–0·6 mg) were dissolved in about 20 μl of 6 M GuHCl. TMK buffer, 0·5 ml, was added, and the solution was incubated at 37°C for 45 min. The solution was then dialysed against TMK buffer at 5–6°C with several changes, the last change normally lasting for at least 12 h. The dialysed samples were clarified at 24,000 *g* at 5°C for 30 min. The UV spectrum of the protein was then obtained. From the UV spectrum and absorptivity data, the concentration of the protein was computed. In those spectra where scattering was observed at 350 nm, a Rayleigh scattering correction, λ^{-4}, was applied as the minimum correction for the scattering and the concentration of the protein computed.

Frictional properties

Sedimentation velocity: Sedimentation velocity experiments were carried out using an epon-filled double sector synthetic boundary cell with a protein concentration yielding an absorbance of 0·5–0·6 at 280 nm in TMK buffer. The $S_{20,\ w}$ values were computed using a Hewlett-Packard 9810A programmable calculator. The log amplified output of the scanner phototube is digitized through an integrating voltmeter and read into the calculator. The details of the procedure adopted are discussed by Inners *et al.* (1978).

Stokes radii and related parameters: Stokes radii of the proteins were determined by gel chromatography using the procedure of Ackers (1967). A Sephadex G-100 column was used, emplyoing 0·1 M KCl TMK buffer, pH 7·36. From the Stokes radius f/f_{min} was calculated.

Molecular weight

Sedimentation equilibrium: Molecular weights (M_r) were determined by sedimentation equilibrium in TMK buffer employing a modification of the high-speed technique of Yphantis (1964). The details of the procedure have been discussed by Aune (1978). Monomer M_r were obtained in 6 M GuHCl containing 0·001 M 2-mercaptoethanol. Data were obtained at several speeds for the mixture of proteins as well as for the homogeneous samples. Plates were read on an LP-6 profile projector equipped with a Nikon stage and micrometers. The calculations of the number, weight and Z-average M_r were performed using a Hewlett-Packard 9810A programmable calculator. Curve-fitting procedures for the monomer, dimer and higher-order complexes along with the mixtures were performed in a Digital DEC-10 computer with a program written in Fortran which employs the procedures discussed previously (Aune and Rohde, 1977).

SDS polyacrylamide gel electrophoresis: The M_r of the proteins was also obtained by the method of SDS-polyacrylamide gel electrophoresis (PAGE) as described by Weber and Osborn (1969) using standard markers. A 12·5% gel with a 1·25% crosslinking was employed and stained with Coomassie brilliant blue.

Results and discussion

The proteins S7 and S19 were tentatively identified based on mobility in gel electrophoresis in the presence of urea and gel chromatography profiles. Confirmation of the identification was based on the correlation coefficients computed from a comparison of their amino acid composition with that already available in the literature (Craven *et al.,* 1969; Kaltschmidt *et al.,* 1970).

The absorptivity at 280 nm of proteins S7 and S19 was found to be $0{\cdot}72_3$ml mg^{-1} cm^{-1} and $0{\cdot}55_5$ml mg^{-1} cm^{-1}, respectively in 15 % acetic acid at 20°C

The individual proteins S7, S9 and S19 were characterized in solution for minimum M_r, M_r in TMK buffer, $S_{20,\ w}$ value, Stokes radius and degree of self-association.

Molecular heterogeneity of a system can be quantitated by analysing data from sedimentation equilibrium experiments. In such experiments the concentration distribution of a single species or a mixture of proteins is given by

$$C(r) = \sum_i C_i(a)\,[\exp\{M_i(1-\bar{v}\rho)\,w^2\,(r^2-r_a^2)/(2RT)\}], \quad (1)$$

where C is the concentration of the ith component at the meniscus, M_i the M_r, $\bar{v}$ is the partial specific volume, ρ is the solution density, w is the angular velocity, r the radial position and r_a the radial position at meniscus, R the gas constant and T the absolute temperature.

The weight average M_r of S7 over the whole cell was computed to be 16,000 ± 500. Figure 1a presents a plot of fringe displacement (in micrometres) *versus* radial position, with a maximum displacement of nearly 900 μm. An earlier report from this laboratory from a different preparation of S7 provided M_r of 20,100 ± 1000 in TMK, 18,700 ± 1000 in GuHCl and 21,000 ± 2100 by SDS-PAGE (Rohde and Aune, 1975). The variability in the M_r observed may be because the strains of *E. coli* from which the protein was purified are different in the two studies. S7 in this study has been isolated from *E. coli* MRE 600 cells and the strain used by Rohde and Aune (1975) was *E. coli* B. The sedimentation equilibrium data for S7 in TMK buffer at 3°C were subjected to curve-fitting procedures. The data fit very well to a system with monomer alone with an average residual of 8 μm. The average residual R is defined by

$$R = \sum_{i=1}^{N} [|\delta_i|/(N-S-1)], \quad (2)$$

where N is the number of data points, S is the number of species in the system, and

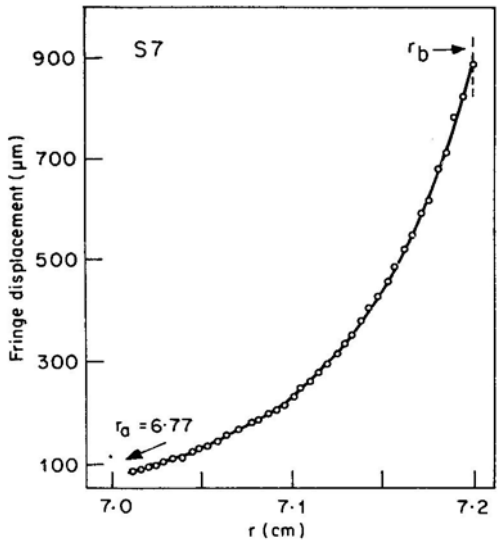

Figure 1a. Fringe displacement (μm) *versus* radial position. Sedimentation equilibrium experiment conditions S7, 0·17mg/ml and 31,410 rpm; solid line is best fit for a single species.

δ is the absolute value of the residual at each point, the residual itself being the difference between the calculated value based on M_r and meniscus concentration and the experimental value. The solid line in figure 1a indicates a monomer fit through the experimental data points. It is clear that no higher-order aggregate is present in significant amounts.

The M_r of S19 determined from sedimentation equilibrium data was 11,000 ± 700 in TMK buffer at 3°C. Figure 1b shows a plot of fringe displacement *versus* radial position for S19 with a displacement of 1100 μm towards the bottom of the cell. From SDS–PAGE a M_r of 13,400 ± 1000 was obtained. The sequence M_r of S19 from *E. coli* K has been reported to be 10,299 (Yaguchi and Wittmann, 1978). The data obtained here from the sedimentation equilibrium experiment is in excellent agreement with the sequence M_r. The sedimentation equilibrium data indicate that the protein exhibited monomeric behaviour over the whole concentration range in the centrifuge cell, suggesting the absence of self-association under the conditions of the experiment. The curve-fitting procedures of the data indicated a monomeric species only with a M_r of 11,000. The solid line in figure 1b indicates the calculated curve for the monomer under the experimental conditions.

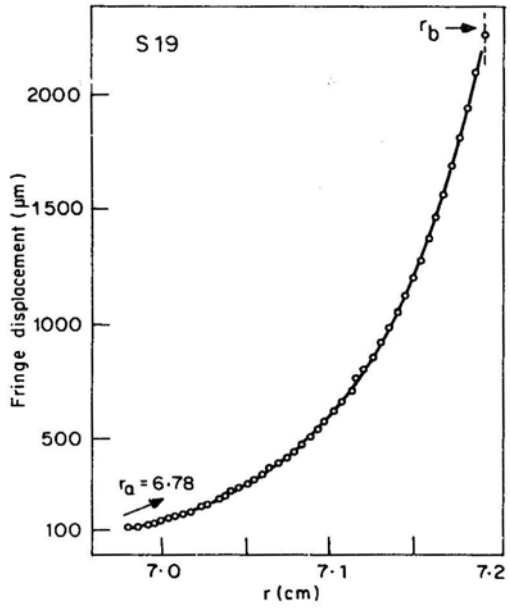

Figure 1b. Fringe displacement (μm) *versus* radial position. Sedimentation equilibrium experiment conditions S19, 0·37 mg/ml, 36,000 rpm; solid line is the best fit for a single species.

The protein S9 has been reported to have a M_r of 14,000 in TMK buffer at 3°C and is monomeric over the concentration range in the sedimentation equilibrium experiment (Prakash and Aune, 1978c). In addition to the minimum M_r and M_r in TMK buffer, the frictional properties of the proteins were examined and are tabulated in table 1. The $S_{20,\ w}$ value of S7 determined at 20°C, 1·66 S, is in agreement with the value of Rohde *et al.* (1975) where S7 was isolated by a slightly

Table 1. Frictional properties of S7 and S19 and sedimentation coefficients of the dimeric and trimeric complexes.

Protein/complex	$S_{20,w}$	f/f_{min}	R_s(A°)	o	f/f_{min}
S7	1·66 ± 0·1	1·35	21·2	0·58	1·27
S19	1·04 ± 0·05	1·64	22·2	0·53	1·50
S7–S9[a]	1·32 ± 0·06	—	—	—	—
S7–S19[b]	1·30 ± 0·24	—	—	—	—
S9–S19[a]	1·61 ± 0·21	—	—	—	—
S7–S9–S19[c]	1·83 ± 0·03	—	—	—	—

[a]Ratio 1:1 by mass. [b]Ratio 1:14 by mass. [c]Ratio 1:1:1 by mass.

different method. Necessarily, f/f_{min} is smaller than previously reported. From both sedimentation and gel chromatography it is observed that S19 appears to be an elongated molecule. The f/f_{min} value computed from both $S_{20,w}$ and R_s turns out to be higher than the typical values of f/f_{min} for globular proteins, which are of the order of 1·20–1·30' in a non-denaturing solvent.

The 3 proteins were refolded as described under materials and methods, dialysed free of GuHCl against TMK buffer, and clarified. After computing concentration, the proteins were mixed in proper ratios, dialysed again against TMK buffer, and analysed for both $S_{20,w}$ values and M_r distribution by sedimentation equilibrium at various speeds.

S7-S19

The proteins S7 and S19 were mixed 1:14 by mass ratio in TMK buffer and data were obtained from both sedimentation velocity and sedimentation equilibrium experiments. The $S_{20,w}$ value for the mixture at a total concentration of 0·5 mg/ml was calculated to be 1·30 ± 0·24 S, which is almost a weight average of the two monomers.

The sedimentation equilibrium data do reveal components of M_r higher than that of either monomer from the point averages of the weight average M_r The plot of the natural logarithm of fringe displacement *versus* r^2 was significantly curved indicating the heterogeneous nature of the system. Figure 1c shows a plot of fringe displacement in μm *versus* radial position for the mixture. The data were subjected to curve-fitting procedures and found to fit very well to a system consisting of 3 species, *i.e.* S7, S19 and S7-S19, instead of just two non-interacting species S7 and S19. These data are therefore consistent with a model where proteins S7 and S19 interact with the simplest stoichiometry of 1:1. The percentage mass as complex formed was calculated according to the equation

$$\% \text{ complex} = 100 \cdot \int_a^b C_3 \, dr^2 / \int_a^b C \, dr^2, \tag{3}$$

by integration from the meniscus *a* to the bottom of the cell *b,* where

$$C = C_1 + C_2 + C_3. \tag{4}$$

The percentage of complex in the 3 experiments varied between 8 and 13 %, which

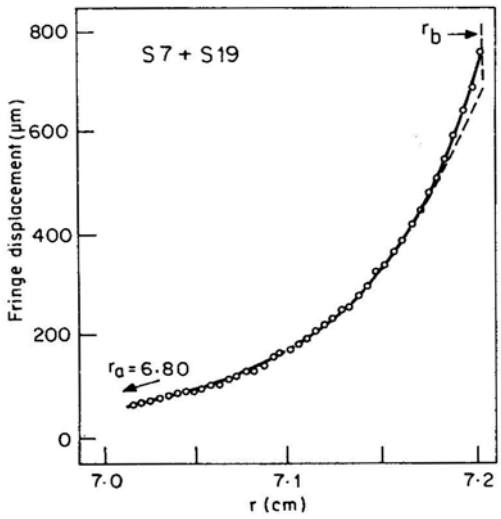

Figure 1c. Fringe displacement (μm) *versus* radial position. Sedimentation equilibrium experiment conditions 1:1·4 ratio (0·25:0·35 mg/ml of each) of S7:S19 at 33,450 rpm at 3°C. The dashed line is the best fit for two species and the solid line is the best fit for 3 species.

is significant enough to recognize the interaction between the two proteins. The data obtained from the curve-fitting performed on sedimentation data for the S7–S19 mixture are given in table 2. These data are utilized for computing the equilibrium constant of association by the equation

$$K = \frac{M_1 M_2}{M_3} \cdot \frac{C_3(a)}{C_1(a) \cdot C_2(a)}, \tag{5}$$

where 1 and 2 represent the proteins that associate to form the complex 3 and C is the concentration at the meniscus. The detailed procedures are described by Aune

Table 2. Parameters determined from curve-fitting of sedimentation data for the mixtures S7–S19, S7–S9, S9–S19 and S7–S9–S19.

Pair	Expt. No.	R^a	R^b	Average residual (μm)	Percentage mass as complex	Equilibrium constt. of association ($\times 10^{-4}$ M)
S7–S19	1	1·4	2·2	4·7	9·0	2·61 ± 0·97
	2	1·4	2·0	10·1	13·0	4·29 ± 3·55
	3	1·4	1·8	12·4	8·0	2·08 ± 1·51
						3·00 ± 2·01[c]
S7–S9	1	1·0	1·1	11·8	15·0	1·61 ± 0·58
S9–S19	1	1·0	1·5	10·0	12·0	6·16 ± 2·0
S7–S9–S19	1	1:1:1	1:1·2:1·1	4·0	6·0 (1–2% of 123,000/ M_r component)	—

[a]Ratio present in the system (see text).[b]Ratio calculated according to Aune (1977). [c]Mean equilibrium constant.

(1977, 1978) and Aune and Rohde (1977). The calculation yields an average equilibrium constant of $3 \pm 2{\cdot}01 \times 10^4$ M^{-1}, which gives a Gibbs free energy of interaction $\Delta G°$ of – 5·7 kcal/mol at 3°C in TMK buffer. These data support the results from crosslinking experiments that the two proteins are together, as has been mentioned earlier.

S7-S9

The proteins S7 and S9 were refolded as mentioned earlier in materials and methods and were mixed in 1:1 ratio by mass. The mixture was analysed by sedimentation velocity and sedimentation equilibrium centrifugation.

The $S_{20,\ w}$ value of the mixture was calculated to be 1·32 ± 0·06 S. The interaction studies by sedimentation equilibrium experiments were performed at 3°C and figure 1d shows a plot of fringe displacement *versus* radial position for the mixture. Curve-fitting of the data was initially performed for two species, S7 and S9, only, but this indicated that a higher M_r species towards the bottom of the cell would have to be considered. The data demanded a complex of even higher M_r than just 30,100 (the sum of S7 and S9 M_r) to be present in the system. After several trials the data were curve-fitted with considerably lower error (in the range .of plate reading) to a system consisting of species of M_r 14,100, 16,000, 30,100 and 120,400. Further, it is to be noted that the data are also consistent with low residuals for components of M_r 14,100, 16,000 and 150,000. No real distinction can be made between these two models or any other comparable combination. The typical data for the S7–S9 system are given in table 2. It can be seen that a complex of M_r 120,400 must be incorporated into the system in order to have meaningful curve-fitting at the

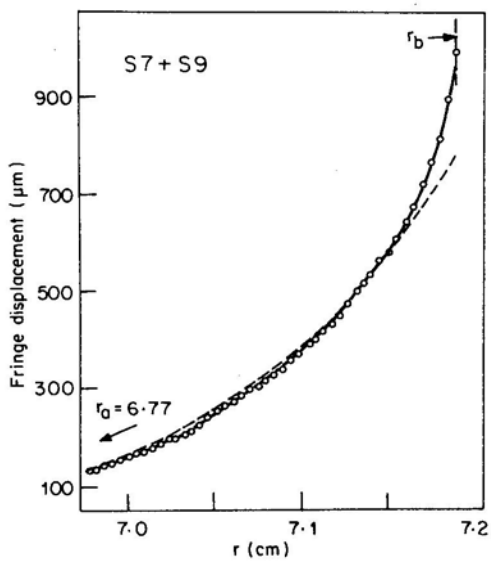

Figure 1d. Fringe displacement (μm) *versus* radial position. Sedimentation equilibrium experiment conditions 1:1 ratio by mass (0·24 mg/ml of each) of S7: S9 at 24,000 rpm at 3°C. The dashed line is the best fit for two species and the solid line is the best fit for the model proposed (see text).

bottom of the cell. Although the molecules are interacting, the system does not lend itself to specific quantitation. The neutron scattering data of Langer *et al.* (1978) indicate close proximity of S7 and S9. The above data support this finding.

S9–S19

The two proteins S9 and S19 were refolded in TMK buffer and mixed in 1:1 ratio by mass as described under materials and methods. The mixture was analysed by sedimentation velocity and sedimentation equilibrium techniques at multiple speeds.

From table 1 it can be seen that S19 has an $S_{20,\,w}$ value of 1·04 ± 0·05 S. The mixture of the two proteins (total concentration ~ 0·7 mg/ml) sediments at 1·61 ± 0·21 S at 20°C. This indicates higher M_r material in the system than just the monomers themselves.

The mixture was examined by sedimentation equilibrium centrifugation employing multiple speeds only at low temperature. Figure 1e shows a plot of fringe displacement *versus* radial position for the mixture. The data were subjected to curve-fitting procedures as before. A logical analysis of the data indicated at least two possible models satisfying the given set of data. The first model predicts a system consisting of the monomers, the complex, and a hexamer of the complex of M_r 150,600. The second model, with the monomers and the hexamer of the complex of M_r 150,600, Fitted the data equally well with the residuals of the curve-fit being

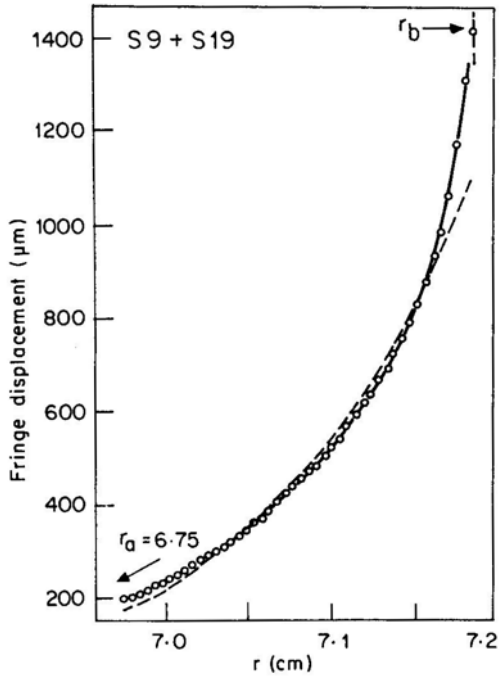

Figure 1e. Fringe displacement (μm) *versus* radial position. Sedimentation equilibrium experiment conditions 1:1 by mass (0·30 mg/ml of each) of S9:S19 at 27,690 rpm at 3°C. The dashed line is the best fit for the two species and the solid line is the best fit for the model proposed (see text).

nearly 10 μm. The data for the S9–S19 interaction are also given in table 2. They indicate that S7 and S19 interact to give a complex in TMK buffer.

S7-S9-S19

Formation of a ternary complex was investigated by mixing S7, S9 and S19 in the ratio 1:1:1 by mass and analysing the data obtained from sedimentation velocity and sedimentation equilibrium experiments at several speeds.

The $S_{20,\ w}$ value for the mixture was 1·83 ± 0·03 S at 20°C (table 1). The mixture was dialysed overnight against TMK buffer. The higher $S_{20,\ w}$ obtained here indicates the presence of a higher M_r complex in the system since none of the monomers has an $S_{20,\ w}$ value greater than 1·65 S. The result and conclusion from this experiment are more of a qualitative nature because of the complexity of the association involved and the heterogeneity of the sample. Sedimentation equilibrium experiments at low temperature provided data which were then analysed by the curve-fitting procedures mentioned earlier. Figure If shows a plot of fringe displacement *versus* radial position for the S7–S9–S19 sedimentation equilibrium experiment. A system with 5 species of M_r 11,000, 14,100, 16,000, 43,300 and 123,300 (a trimer of the complex of the monomers) provides a residual error of 4 μm and proper mass ratio. However, this extreme 5-species fit leaves the system containing only 1–2% of the mass as a high M_r complex. Intermediate, more complex systems with simple trimers as well as high M_r complex suggest 4% of the mass involved in interaction. Even though the amount of the complex cannot be defined, the presence of it, even at low levels, is essential for meaningful curve-fitting from the sedimentation equilibrium data.

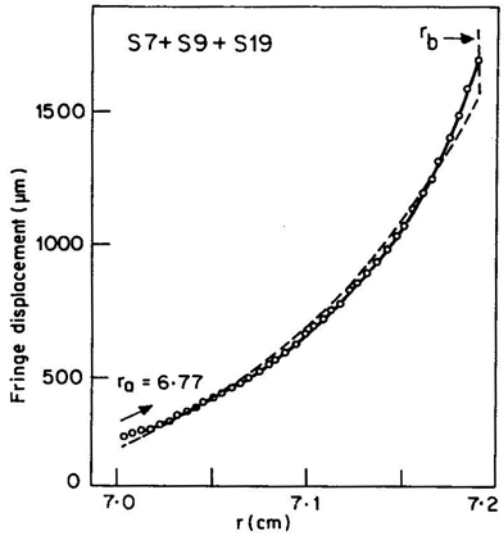

Figure 1f. Fringe displacement (μm) *versus* radial positions. Sedimentation equilibrium experiment conditions 1:1:1 ratio by mass (0·46 mg/ml of each) S7: S9: S19 at 26,000 rpm at 3°C. The dashed line is the best fit for 3 species and the solid line is the best fit for the model proposed (see text).

The results indicate formation of complexes between the proteins S7, S9 and S19 both in pairs and as a ternary combination. The nature of these complexes cannot be identified with the present data.

Table 3 gives the energy of interaction for some pairs of 30S ribosomal proteins. The data indicate that even though S7 and S19 interact to a considerable degree, the interaction is not as strong as that between S3 and S5 or between S5 and S10, but is stronger than that between S4 and S5.

Table 3. Energy of interaction for some pairs of 30S ribosomal proteins.

Pair	Interaction	ΔG° (K cal/mol)
S2–S3[a]	–	—
S3–S4[b]	+	−5·1
S3–S5[c]	+	−7·3
S4–S5[b]	+	−4·8
S4–S9[e]	+	−5·8
S4–S20[b]	–	—
S5–S10[a]	+	−7·4
S6–S18[d]	+	−6·0
S6–S21[e, f]	+	−6·6
S7–S9	+	−5·3
S7–S19	+	−5·7
S9–S19	+	−6·0
S18–S21[b]	+	−5·6

[a]Rohde and Aune (1975). [b]Aune (1977). [c]Rohde *et al.* (1975). [d]Prakash and Aune (1978a). [e]Prakash and Aune (1978b). [f]Prakash and Aune (1978c).

The results of the present studies indicate that the proteins, S7, S9 and S19 are monomeric in TMK buffer at 3°C and S9 aggregates at higher temperatures. The protein S9 behaves differently in the presence of S7 and S19. The behaviour of S9 in the presence of S4, although different, still permits an evaluation of the equilibrium constant of association (Prakash and Aune, 1978c). When S7 and S19 were mixed and the mixture analysed by sedimentation equilibrium experiments, 8–13% complex formation was observed, there were no higher-order complexes, and the energy of interaction was computed to be – 5·7 kcal/mol. The presence of S9 in the system, S4–S9 (Prakash and Aune, 1978c) S7–S9, S9–S19 or S7–S9–S19, makes the complex of the proteins to assume a very high M_r. The data suggest an unusual role for the protein S9. Since the assembly process must contend with these thermodynamic states it would appear that protein S9 is a dynamic 'glue' in the 30S ribosome. It is more interesting that in order to restrict complex formation of S9 with a single protein, nature has probably placed it amidst a number of other 30S ribosomal proteins, amongst them S4, S7 and S19, with which it can complex. Compared to other models the significance of S19 in the proximity of S4, S7 and S9 is to be noted. In view of the models of Nomura and Held (1974) and Cornick and Kretsinger (1977) the interaction of proteins S5, S8 and S10 with S9 should throw more light on the above system and would help in a better understanding of the topography of the 30S subunit from an energetics point of view. These data, along

with other data on the 30S subunit would help ultimately in building an energy map of the small subunit of the *E. coli* ribosome.

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