

Immunoprecipitation of 70S, 50S and 30S ribosomes of *Escherichia coli*

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Abstract. Antibodies were raised in rabbits against 70S ribosomes, 50S and 30S ribosomal subunits individually. Purified immunoglobulins from the antiserum against each of the above ribosomal entities were tested for their capabilities of precipitating 70S, 50S and 30S ribosomes. The observations revealed the following: (i) The antiserum (IgG) raised against 70S ribosomes precipitates 70S ribosomes completely, while partial precipitation is seen with the subunits, the extent of precipitation being more with the 50S subunits than with 30S subunits; addition of 50S subunits to the 30S subunits facilitates the precipitation of 30S subunits by the antibody against 70S ribosomes. (ii) Antiserum against 50S subunits has the ability to immunoprecipitate both 50S and 70S ribosomes to an equal extent. (iii) Antiserum against 30S subunits also has the property of precipitating both 30S and 70S ribosomes. The differences in the structural organisation of the two subunits may account for the differences in their immunoprecipitability.

Keywords. Ribosomes; ribosomal subunits; antibody; immunoprecipitability.

Introduction

The topography of ribosomal proteins has been investigated by a number of elegant experimental approaches including immunology coupled with electron microscopy (for review, see Stouffer and Wittmann, 1977). The availability of specific antibodies to each of the 54 ribosomal proteins from *Escherichia coli* ribosome was a prerequisite for these studies. Apart from antisera to two proteins pairs L7 and L12 as well as S20 and L26), no immunological cross-reaction has been found between the different antisera raised individually against all the proteins of the ribosome. Some immunological approaches towards the study of the structure of the ribosome have been made in this laboratory (Das and Burma, 1979). During these preliminary investigations antiserum was raised in rabbits against intact *E. coli* 70S ribosome and it was observed that such antiserum efficiently precipitates 70S as well as 50S ribosomes but not 30S ribosomes. Subsequently antisera were raised against individual ribosomal subunits and detailed studies on the immunoprecipitation of the two subunits were carried out. These studies will be presented here. Similar observations have been made with rat liver ribosome (Stouffer *et al.*, 1978).

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Materials and methods

The sources of the materials used have been mentioned in an earlier publication (Das and Burma, 1979). Sulphur-35 [^{35}S] as sulphuric acid (carrier-free) in dilute HCl was the product of Bhabha Atomic Research Centre, Trombay, Bombay.

The immunisation of rabbits, isolation of immunoglobulin (IgG) from antiserum and methods of immunoprecipitation etc. have already been described (Das and Burma, 1979).

Preparations of [^{35}S]-labelled ribosome and its subunits

The method described by Sun *et al.* (1974) was followed for growing *E. coli* in [$^{35}\text{SO}_4^{2-}$]-containing medium. Under this condition, the cells incorporated 60-70% of the radioactivity. These radioactive cells (2 g wet weight) were mixed with nonradioactive cells (5 g) for preparing [^{35}S]-labelled ribosomes. Ribosomes were prepared as described earlier (Datta and Burma, 1972). The final ribosomal preparation had a specific activity of 8.2×10^4 counts/min/ A_{260} unit. The 50S and 30S subunits were isolated from (^{35}S)-labelled ribosomes following the method described by Godson and Cos (1970). Final preparations of 30S and 50S subunits had specific activities of 7.3×10^4 and 5.1×10^4 counts/min/ A_{20} unit, respectively.

Immunoprecipitations of radioactive ribosomes by varying amounts of IgG or antisera

The method of Chu *et al.* (1976) was used with some alterations. [^{35}S]-labelled 70S, 50S and 30S ribosomes (200 μ g containing $2-3 \times 10^4$ counts/min) were individually mixed with increasing amount of IgG or antisera, as the case may be, in presence of 0.05 M Tris-HCl, pH 7.4, 0.01 M magnesium-acetate and 0.5 M NH_4Cl in a total volume of 0.5 ml and the mixture incubated at 37°C for 30 min and subsequently at 0°C for 1 h. The precipitate obtained by centrifugation at 15,000 g for 15 min at 0°C was washed twice with 0.05 M Tris-HCl, pH 7.4, 0.5 M NH_4Cl and 1% (v/v) Triton XX100 (1 ml) and finally suspended in 4M urea containing 50% acetic acid and 1% Triton XX100 (0.25 ml). Aliquots (0.1 ml) were added to 5 ml of Bray's solution (Bray, 1960) and counted in a Nuclear Chicago liquid scintillation counter. Percentage of ribosomes precipitated was calculated from the radioactivity in the immunoprecipitate.

Results

Immunoprecipitation of [^{35}S]-labelled 70S ribosome and its subunits by IgG isolated from the antiserum raised against 70S ribosome

It is evident from the results presented in figure 1a that there is more immunoprecipitate formed with the increasing amount of IgG used for a fixed amount (200 μ g) of 70S or 50S or 30S ribosomes. However, a wide difference is found between the extents of precipitation of ribosomes and their subunits. For example, 90 to 100% of the 70S ribosome is precipitated by the addition of 1 to 2 mg of IgG. In case of 30S ribosome, however, only one third or less amount is precipitated by the addition of similar amount of IgG; actually 0.25 mg IgG is sufficient for the purpose. In case of 50S ribosome about 60% of the subunit is precipitated with the addition of 1 mg of IgG. Doubling the amount of IgG leads to a somewhat less precipitation (50%).

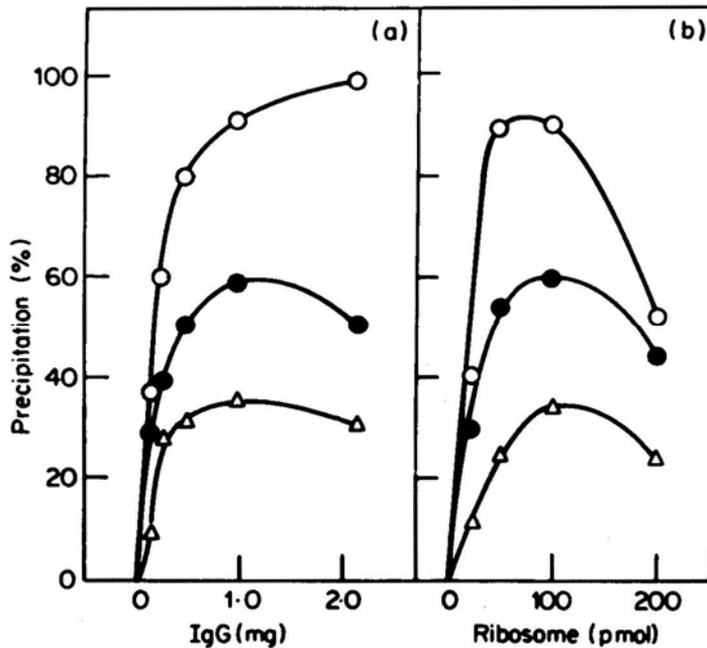


Figure 1. Immunoprecipitation of $[^{35}\text{S}]$ -labelled 70S ribosome and its subunits by IgG isolated from the serum raised against 70S ribosome. $[^{35}\text{S}]$ -labelled 70S, 50S and 30S ribosomes ($200\ \mu\text{g}$, 2.5×10^4 counts/min) were incubated with different amounts of IgG (A) and a fixed amount of IgG (0.75 mg) was incubated with different amounts of $[^{35}\text{S}]$ -labelled 70S, 50S and 30S ribosomes (B). The percentage of precipitation was determined from the radioactivity of the precipitate formed. The details have been described under Materials and methods. — Δ — 30S; — \bullet — 50S; — \circ — 70S.

Similar observation is made when a fixed amount of IgG (0.75 mg) was added to varying amounts of ribosomes (figure 1b). Under this condition 50-100 pmol of ribosomes are precipitated to the extent of 90%. However, when excess ribosome is added there is much less precipitation, as expected. Under the same condition, however, only 60% of 50S ribosomes is precipitated. There is a slightly less amount of precipitation with the addition of increasing amount of the same subunit. Again the precipitation of the 30S subunit is comparatively much less and only 30-35% of the 30S subunit is precipitated under identical conditions. These results clearly indicate that the antibody raised against 70S ribosome behaves differently towards 50S and 30S ribosomes so far as the extent of precipitation is concerned.

Immunoprecipitation of 70S ribosome and its subunits by varying amounts of antiserum raised against 50S ribosome

The immunoprecipitating capacity of antibodies raised separately against the individual subunits was tested against 70S ribosome and its subunits. Since it was known earlier that in the case of 70S ribosome, the behaviour of the antiserum and that of IgG prepared from the antiserum are practically the same, (data not shown), the antiserum was used directly for precipitation of ribosomes in these experiments. The results obtained with antisera raised against 50S ribosome are shown in figure 2.

When a fixed amount ($200\ \mu\text{g}$) of ribosome is used, both 70S and 50S ribosomes are precipitated to the maximum extents (70-75%) by 0.05 to 0.10 ml of the antisera

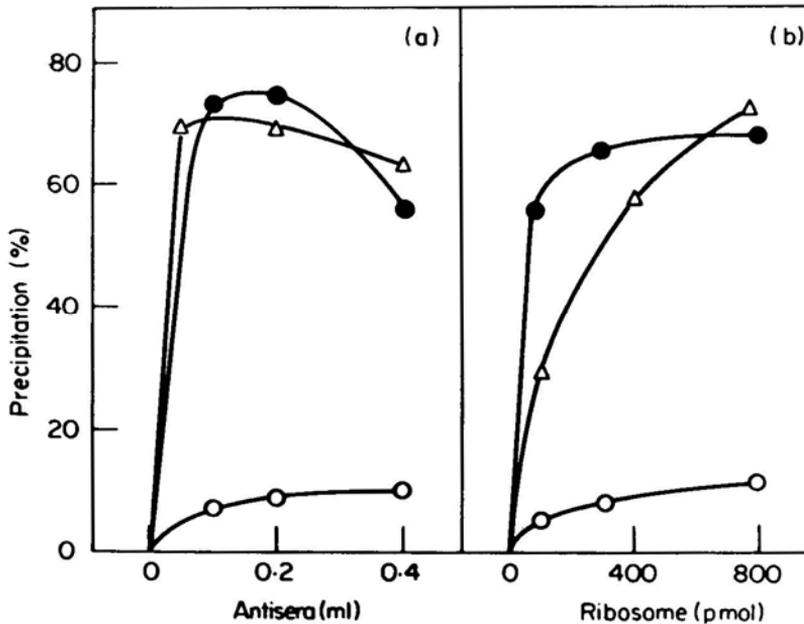


Figure 2. Immunoprecipitation of ^{35}S -labelled 70S ribosome and its subunits by antiserum raised against 50S ribosome. ^{35}S -labelled 70S, 50S and ribosomes ($200\ \mu\text{g}$, 2.5×10^4 counts/min) were incubated with different amounts of antiserum (A) and a fixed amount of antiserum (0.1 ml, 7 mg) was incubated with different amounts of ^{35}S -labelled 70S, 50S, and 30S ribosome (B). The percent precipitation was calculated from the radioactivity in the immunoprecipitate as described under Materials and methods. — O — 30S; — Δ — 50S; — \bullet — 70S

(figure 2b). Increasing the amount of antiserum does not lead to any further increase in the immunoprecipitate on the other hand, there is slightly less amount of precipitate formed. However, there is small amount of precipitate formed with the 30S ribosome. Since the 50S ribosome used for immunisation had slight contamination of 30S ribosome (and vice versa), this was not quite unexpected. Similar observation was made when a fixed amount of antiserum (0.1 ml) and varying amount of ribosomes were used (figure 2b). This amount of antiserum is capable of precipitating 70% of either 70S or 50S ribosome when 800 pmol of either ribosome are used. With the use of lesser amount of ribosome, lower amount of ribosome is precipitated, as expected. Again as in the earlier case, 5-10% of 30S ribosome is precipitated by the antiserum raised against the 50S ribosome. This may be due to the cross-contamination of the preparations, as discussed above.

Immunoprecipitation of ^{35}S -labelled 70S ribosome and its subunits by antiserum raised against 30S ribosome

The results obtained with varying amounts of antiserum raised against 30S ribosome have been presented in figure 3a and those obtained with varying amounts of ribosomes have been presented in figure 3b. There is increasing amount of immunoprecipitation of 70S and 30S ribosomes with the increasing amount of antiserum used. With the addition of 0.1 ml of the antiserum to $200\ \mu\text{g}$ of 70S ribosome there is about 70-75% precipitation. However, on addition of double this amount, somewhat less amount

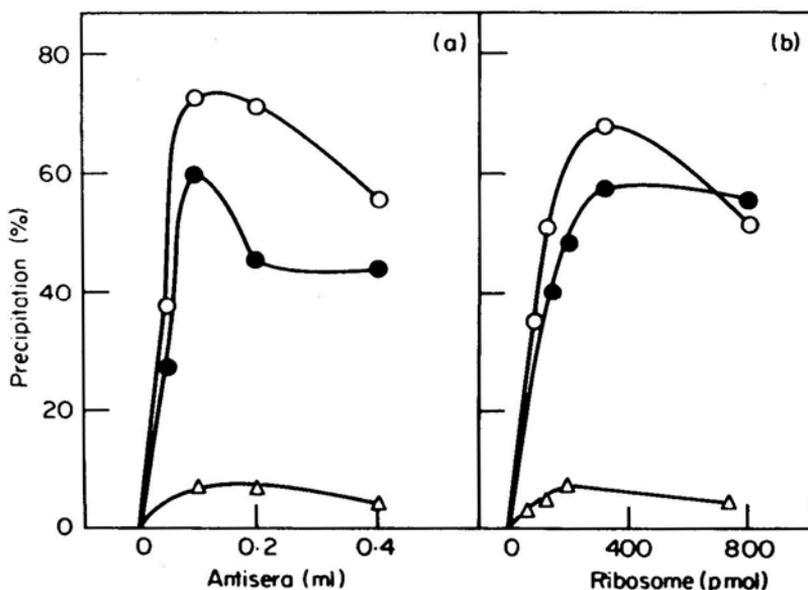


Figure 3. Immunoprecipitation of $[^{35}\text{S}]$ -labelled 70S ribosome and its subunits by antiserum raised against 30S ribosomes. Incubations were done as described in the legend to figure 2 with the difference that the antiserum raised against 30S ribosome was used. The details have been mentioned in Materials and methods. —○— 70S; —●— 30S; —△— 50S

of precipitate is formed. With the 30S ribosome, maximum precipitation (60%) was obtained with 0.1 ml of antiserum. With increasing amounts of antiserum lesser quantity of precipitate (40-45%) is formed. Again there is small but significant amount of immunoprecipitation of the 50S ribosome, possibly due to cross-contamination. Similar results were obtained when a fixed amount (0.1 ml) or antiserum and varying amount of ribosomes were used (figure 3a). Maximum precipitation obtained in case of 70S and 30S ribosomes is 55-65%.

Effect of 50S ribosome on the immunoprecipitation of 30S ribosome with IgG against 70S ribosome

It is clear from the results presented in figure 1 that the 30S ribosome is not as efficiently precipitated as the 50S ribosome. In order to check whether the 50S ribosome will help in the precipitation of 30S ribosome, non-radioactive 70S ribosome or 50S subunits were added and further incubated. It is clear from the results presented in figure 4 that the addition of unlabelled 70S ribosome does not help in the precipitation of 30S subunit by two different concentrations of IgG against 70S ribosomes. However, addition of unlabelled 50S subunit enhances the precipitation of 30S subunit to a considerable extent.

Discussion

It is clear from the results presented above that the 50S ribosome of *E. coli* is more efficiently precipitated than the 30S ribosome by the antisera raised against either intact 70S ribosome or individual subunits. As mentioned in the introduction, this difference in behaviour of the two subunits has also been observed in case of rat

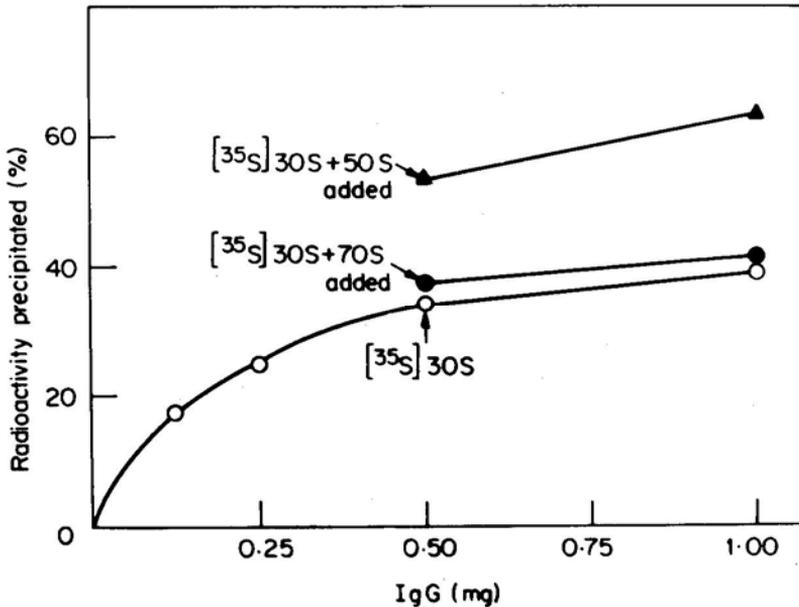


Figure 4. Effect of addition of unlabelled 70S and 50S ribosomes on the immunoprecipitation of [³⁵S]-labelled 30S-ribosome by IgG isolated from the antiserum raised against 70S ribosome. The immunoprecipitation of [³⁵S]-labelled 30S ribosomes by IgG was carried out as described in the legend to figure 1. Same amount of nonradiative 70S ribosome or 50S ribosome was added at the point indicated by the arrows.

liver ribosomes, although no explanation has been provided (Stoffer *et al.*, 1978). One of the reasons in case of *E. coli* ribosomes could be that the proteins of 50S ribosome are more antigenic than those of 30S ribosome but this seems unlikely from the detailed studies carried out by Stoffer and his coworkers (Stoffer *et al.*, 1973; Morrison *et al.*, 1977). It may also be argued that 50S ribosome is double in size than the 30S ribosome hence more efficiently precipitated but still the latter is a giant molecule composed of 21 proteins, therefore the size would hardly matter. One other argument could be that more soluble complex is formed in case of 30S ribosome. However, ultracentrifugation experiments (results not presented) do not corroborate this. On the other hand, it may be speculated that the 30S ribosome has a more compact structure than the 50S ribosome, the better accessibility of the proteins in the larger ribosome helps in its efficiency precipitation. The overall structural organisation of the two subunits may also be quite different (Burma, 1979). In many respects the two ribosomal subunits behave differently, for example, 50S ribosome is more susceptible to the action of RNase I than 30S ribosome (Datta and Burma, 1972); however, when a part of the structure of 50S ribosome is removed by the action of RNase I, the 50S core particle behaves like 30S ribosome (Raziuddin *et al.*, 1979). Further, the intercalation of the dye, ethidium bromide into RNA of the 50S ribosome is very much dependent on the concentration of Mg²⁺, it is much less dependent in the case of 30S ribosome (Burma, *et al.* 1979). There is also considerable difference in the action of trypsin on the two subunits, the larger ribosome being more susceptible than the smaller one (Ali, 1978). Such difference is also observed in the action of formaldehyde on the two subunits (Chatterji *et al.*, unpublished observations). Very recently it has been observed that monovalent cations have

tremendous influence on the structure of 50S ribosome whereas the effect is very small in case of 30S ribosome (Raziuddin, unpublished observations). Considering these observations along with the that made in this paper it appears that there is a basic difference in the structural organisation of the two subunits and this may account for the inefficiency precipitation of 30S ribosome in comparison with the 50S ribosome.

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