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Alteration of the structure of the *Escherichia coli* ribosomes on treatment with Fab fragment of immunoglobulin raised against the ribosomes

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Abstract. Rabbits were immunised against *Escherichia coli* ribosomes and the partially purified immunoglobulin G fraction had maximum ability to precipitate the ribosomes as well as the extracted ribosomal proteins. By digestion of immunoglobulin G with papain, monovalent Fab fragments were produced. The 70 S ribosome and its subunits (50 S and 30 S) were separately treated with Fab and then tested in the kinetic assay of degradation of ribosomes by ribonuclease I at various Mg^{2+} concentrations. Treated ribosomes and their subunits were degraded at faster rates than the nontreated ones; the rates in both the control and the treated cases were dependent on the concentration of Mg^{2+}. These results indicate the unfolding of the structure of the ribosome on treatment with antibody fragments, which may be due to the weakening of the interaction between rRNAs and ribosomal proteins.

Keywords. Ribosomes; ribosomal subunits; antibody; immunoglobulin; Fab; ribonuclease I.

Introduction

It has been shown in this laboratory that the treatment of *Escherichia coli* ribosome with intercalating and non-intercalating dyes (Suryanarayana and Burma, 1975; Sinharay *et al*., 1977), bisulphite (Das, 1977), thiol reacting reagents (Suryanarayana, 1975) and trypsin (Ali, 1978) lead to the unfolding of the structure of the ribosome. Immunoelectronmicroscopy is being extensively used in the study of the structure of the ribosome (for review see Stoffler and Wittmann, 1977). In these studies, immunoglobulin G (IgG) purified from the antiserum raised against a specific protein of the ribosome, is reacted with the ribosome and from the electronmicroscopic picture of the treated ribosome, the location of that protein is visualised. It was therefore of interest to see whether the treatment of ribosome with IgG induces any alteration in the ribosome structure. It was not expected to detect any structural alteration by directing antibody against only one protein, and therefore antibody was raised against all the proteins (Stofller *et al*., 1973; Morrison.

et al., 1977). But the inherent difficulty of using IgG from such antiserum lies in the precipitation of the ribosome. Therefore, the Fab fragment of IgG which has one antigen binding site per molecule which did not precipitate the ribosome was used in the present study. The conformational changes in the ribosome was monitored using the susceptibility of ribosomes to degradation by ribonuclease I (RNase I) (Datta and Burma, 1972).

Materials and methods

Materials

Poly A was from Miles Laboratories, USA; sephadex G-200 from Pharmacia, Uppsala, Sweden; Freund's adjuvants (complete and incomplete) from Difco Laboratories, Detroit, MI, USA; crystalline papain (E.C. 3.4.4.10) was from Sigma Chemical Co., St. Louis, Mo, USA. All other chemicals were of analytical reagent grade. RNase I (E.C. 2.7.7.17) was purified from *Salmonella typhimurium* according to the method of Datta and Burma (1972).

Methods

Preparation of E. coli ribosomes, ribosomal proteins and rRNAs: *E coli* MRE 600 (RNase I^-) was grown in large batches at 37° C in synthetic medium as described by Rao and Burma (1971). Ribosome was prepared by ultracentrifugation from the extract of *E. coli* obtained by grinding with alumina (double the wet wt of cells) and washed twice with 1 M NH_4C1. S^{35}-labelled ribosome was prepared from cells grown in $^{35}SO_4^{2-}$-containing medium according to the method described by Sun *et al*., (1974). The ribosomal preparation had the specific activity of 3–5 $\times$ 15^5 cpm/A_{260} unit. Ribosomal proteins were prepared by the method described by Spitnik-Elson (1965). Ribosomal RNAs were prepared from ribosome by repeated phenol extraction and ethanol precipitation.

Immunisation of rabbits with ribosomes: Rabbits were injected intradermally at 4 sites with 5 mg of *E. coli* ribosomes in 0·02 M Tris-HCl, pH 7·4, 10 mM magnesium acetate and 0·03 M KCl mixed with an equal volume of Freund's adjuvant (complete) and on the 21st day with the incomplete adjuvant. Rabbits were test-bled on the 30th day from the ear vein and booster injections (4–5) were given every 4–5 weeks. They were bled after 7–10 days following the booster injection. The immunoprecipitation was carried out according to the method of Stoffler and Wittmann (1971), using the antibody raised as described above, and ribosomes, ribosomal proteins and rRNAs as antigens.

Treatment of ribosomes with Fab: *E. coli* ribosomes or 50 S or 30 S subnit (2·5 A_{260} units) in 0·5 ml (total volume) was treated with 1·25 mg Fab in presence of 0·02 M Tris-HCl, pH 7·4 and 0·002 M magnesium acetate. This mixture was incubated at 37° C for 1 h. The ribosomes incubated under the same condition but in absence of Fab was taken as control (untreated).

Measurement of the degradation of ribosomes by RNase I: Degradation of ribo somes and its subunits by RNase I was followed by measuring the increase in

absorbancy at 260 nm in a PMQ II Zeiss spectrophotometer as described by Suryanarayana and Burma (1975). In each experiment 0·5 A^{260} unit of ribosomes or 50S or 30S sub-unit and 2 units of RNase I were used.

Immunoprecipitation of ribosomes, ribosomal proteins and rRNAs by antisera raised againstribosomes : It was not possible to immunise the rabbit against ribosomal proteins as these proteins could be kept in solution only in presence of high concentration (8 M) of urea. Since the antiserum was raised against ribosomes, both rRNAs and proteins may be involved in immunoprecipitation. Therefore the antiserum was tested separately against ribosomes, ribosomal proteins and rRNAs by adding varying amounts of antiserum against a fixed amount of antigen (figure 1). The precipitation was maximal with intact ribosomes, as expected. Ribosomal proteins were also precipitated but to somewhat lesser extent. rRNAs were precipitated to the least extent. The antiserum was also tested by adding varying amounts of the antigen to a fixed amount of antiserum (Heidelberger curves).

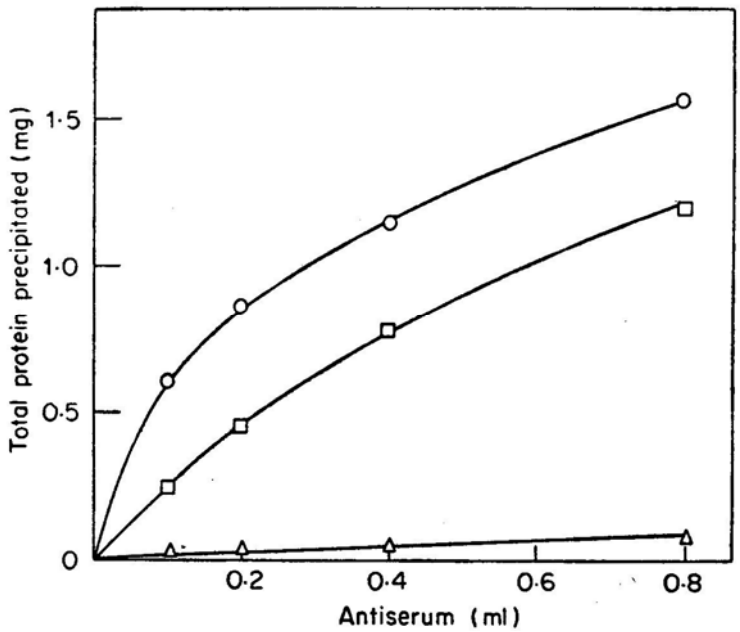

Figure 1. Immunoprecipitation of *E. coli* ribosomes, ribosomal proteins and rRNAs with antiserum against *E. coli* ribosomes.
Immunoprecipitation was carried out according to the method of Stoffler and Wittmann (1971) using a fixed amount (150 μg) of antigen (ribosomes or ribosomal proteins or rRNAs and varying amounts of antiserum). O Ribosome; □ ribosomal proteins: △ r RNAs.

Preparation of immunoglobulin : The antiserum was precipitated with ammonium sulphate (40% saturation) and IgG and IgM fractions were separated on a sephadex G–200 column. IgG fraction precipitated the ribosomes to the maximum extent while IgM fraction had very little activity. This indicates that the precipitating antibody against the ribosome is of IgG type. The IgG fraction was

concentrated by ammonium sulphate precipitation; the ammonium sulphate in the precipitate was removed by dialysis.

Preparation of Fab: Monovalent fragments of immunoglobulin were prepared by treating the latter with papain according to the method of Putnam *et al.* (1962) The fragmentation of IgG was ascertained by M_t determination using gel nitration. The fragment had a M_t of 50,000, corresponding to Fab and Fc portions of the IgG.

Before using the fragments, the reaction mixture was dialysed vigorously at 0° C for 24 h against distilled water with frequent changes, to inactivate papain. It was found that after such extensive dialysis, there was no detectable papain activity in the reaction mixture as assayed by casein hydrolysis (Arnon, 1970) The reaction mixture was finally dialysed. for 3 h against 500 ml of 0·002 M Tris-HCl, pH 7·4.

Assay of Fab: ^{35}S-Labelled ribosome (200 μg, 2 × 10^4 cpm) was first allowed to react with Fab (1·5 or 3 mg) at 37° C for 1 h and then IgG (1·5 mg) was added and the incubation was continued at 37° C for another hour. There was no

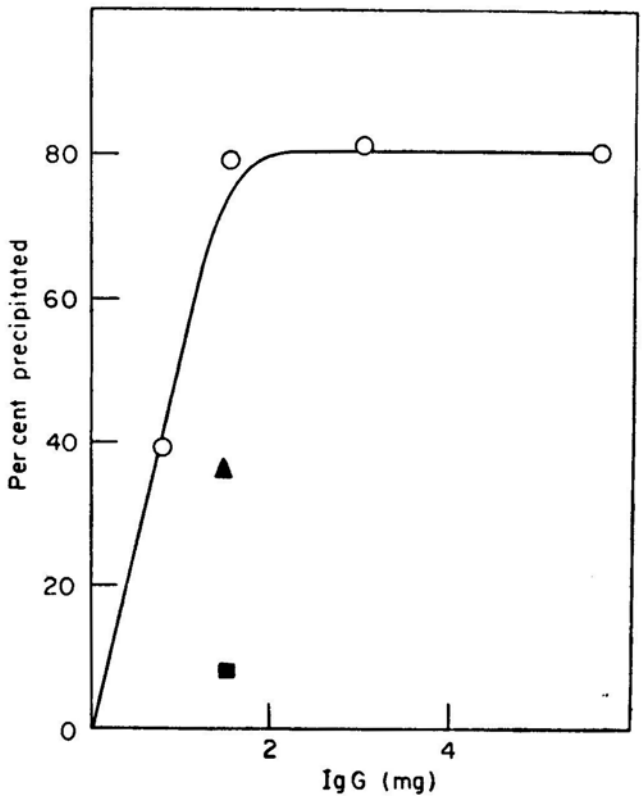

Figure 2. Assay of Fab.

The curve represents a typical immunoprecipitation of control ribpsomes (^{35}S-labelled) by increasing amounts of IgG. Other two points, marked ▲ and ■ indicate per cent of ribosomes precipitated by 1·5 mg IgG when ribosomes were pretreated respectively with. 1·5 mg and 3 mg Fab fraction (containing Fc as well).

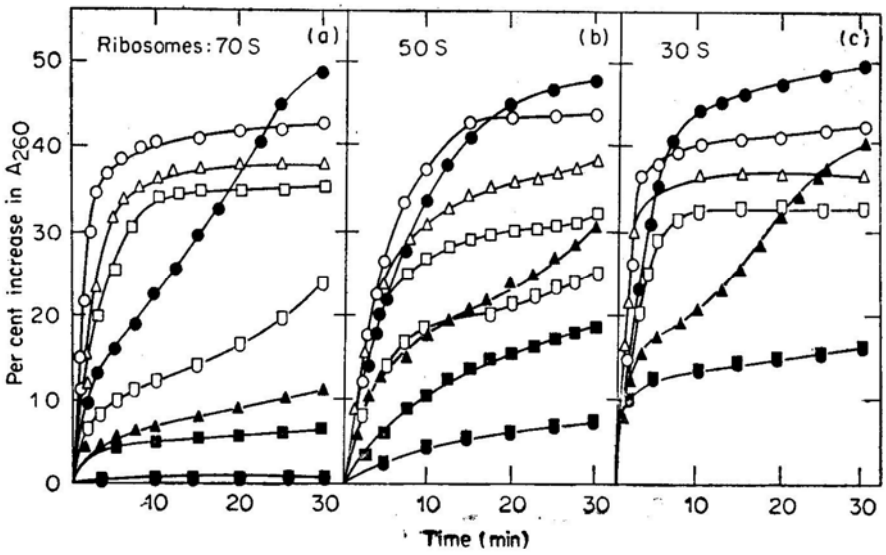

Figure 3. Degradation of Fab-treated ribosomes by RNase I.
Ribosomes, 70S and 50S and 30 S subunits were individually treated with Fab and then degraded by RNase I as described under materials and methods.

(**a**) 70S ribosome

● untreated ; O treated ; assayed at 0.2 mM Mg^{2+}
▲ untreated ; Δ treated ; assayed at 0.4 mM Mg^{2+}
■ untreated ; □ treated ; assayed at 0.5 mM Mg^{2+}
◆ untreated ; ◇ treated ; assayed at 1.0 mM Mg^{2+}

(**b**) 50S ribosome :

● untreated ; O treated ; assayed at 0.2 mM Mg^{2+}
▲ untreated ; Δ treated ; assayed at 0.4 mM Mg^{2+}
■ untreated ; □ treated ; assayed at 0.5 mM Mg^{2+}
◆ untreated ; ◇ treated ; assayed at 1.0 mM Mg^{2+}

(**c**) 30S ribosome :

● untreated ; O treated ; assayed at 0.2 mM Mg^{2+}
▲ untreated ; Δ treated ; assayed at 0.4 mM Mg^{2+}
◆ untreated ; ◇ treated ; assayed at 1.0 mM Mg^{2+}

precipitate at the end of the first incubation, as expected, for the reaction with monovalent Fab fragment. The percentage of ribosomes precipitated, at the end of the second incubation was estimated. The idea behind this experiment was that if some of the antigenic sites of ribosomes are already complexed with Fab, those will not be available to react with IgG subsequently added, thus there will be an inhibition of the immunoprecipitation of ribosomes by IgG. It is clear from figure 2 that the percentage of ribosomes, precipitated by 1·5 mg IgG preparation, decreased from 77 to 36 by 1·5 mg Fab preparation, (containing Fc as well) and to 8% by 3 mg of the same preparation. Thus there is increased inhibition of

immunoprecrpitation by increasing amounts of Fab, confirming the antigen binding activity of the preparation.

Results

Effect of treatment of 70 *S*, 50 *S and* 30 *S ribosomes with Fab*

The 70 S ribosomes and 50 S and 30 S subunits were separately treated with the Fab preparation. The degradation by RNase I of the treated ribosomes and subunits and the corresponding nontreated ones, was separately followed at different concentrations of Mg^{2+}. The increase in A_{260} at various time intervals was measured (figure 3). In each case, Fab-treated ribosomes or subunits are degraded at a higher rate than the corresponding untreated ones. The difference in the rates of degradation is dependent on the concentration of Mg^{2+} and is usually greater at higher Mg^{2+} concentrations. It should be mentioned here that at lower concentrations of Mg^{2+}, 50 S, ribosomes are degraded at a very fast rate unlike in the case of 70 S ribosomes and 30 S subunits. Therefore, in order to have a measurable rate, considerably higher concentrations of Mg^{2+} (up to 10 mM) have been used. When the ribosome is incubated with Fab alone under the conditions of degradation by RNase I, there is no increase in A_{260} at least for 30 min This indicates that the Fab fragment is not contaminated with a nuclease.

The time course of degradation in most cases is biphasic and sometimes multiphasic The rates alter considerably after initial increase of absorbancy (A_{260}). This could be due to the compactness and complexity of the structure of the ribcsome. At low Mg^{2+} concentrations and specially on treatment with Fab, the degradation proceeds at a fast rate till the hydrolysis is more or less complete.

Discussion

It was difficult to immunise rabbits with ribosomal proteins due to the poor solubility of the ribosomal proteins except in the presence of 8 M urea. The rabbits were immunised with intact ribosomes. Although it was not expected that the ribosome will remain intact in the circulation, the antiserum was found to be mainly effective against ribosomal proteins and not rRNAs (figure 1). From the work of Stoffler *et al.* (1973) and Morrison *et al.* (1973) it can be assumed that antibodies weie most probably produced against all ribosomal proteins. As IgG caused precipitation of ribosomes, Fab fragments were used and an assay method for testing the activity of Fab fragment was developed (figure 2).

Intercalating and non-intercalating dyes (Suryanarayana and Burma, 1975; Sinharay *et al*., 1977) which alter the structure of rRNA, thiol reacting reagents (Suryanarayana, 1975) which reacts with proteins, bisulphite (Das, 1977) which modifies ribosomal proteins, unfold the structure of the ribosomes. The treatment of 70 S, 50 S ar 30 S ribosomes with Fab resulted in the enhanced rate of degradation by RNase I (figure 3). As expected, Mg^{2+} has considerable influence on the rates of degradation and the effects are not the same in the case of 50 S and 30 S subunits. It is clear from these data that Fab treatment causes structural alteration in ribosomes as well as in its subunits. I is not known whether some proteins

are released from the ribosomes treated with Fab. As a result of the conformational change induced by Fab, the structure of ribosomes and its subunits become unfolded and hence are more susceptible to the hydrolysis by RNase I. This finding supports the earlier observations from this laboratory that whenever ribosome structure is distorted, it becomes more susceptible to hydrolysis by RNase I

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