# ISOLATION OF SERUM ALBUMIN-SYNTHESIZING POLYSOMES FROM RAT LIVER

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SUMMARY

The procedures for the purification of rat liver polysomes synthesizing serum albumin was developed, employing the quantitative precipitin method with rat serum albumin as a carrier and its antibody, and ribonuclease inhibitor from rat liver. The addition of ribonuclease inhibitor to polysomes during the incubation with antibody was found to prevent their degradation. Under these conditions, about 12 % of the membrane-bound polysomes of rat liver was found in the specific precipitate of serum albumin and its antibody, while a negligible amount of free polysomes was precipitated. It is concluded that polysomes synthesizing serum albumin are isolated by this method.

## INTRODUCTION

It has become well known that in rat liver cells, there are two kinds of polysomes, free and membrane-bound polysomes. Several reports, including those of authors, have been published concerning with the functional differences between these two kinds of polysomes. For example, it was shown by both *in vivo* and *in vitro* experiments that serum albumin was synthesized only on membrane-bound polysomes (1-3). On the contrary, the site of ferritin synthesis was shown to be on free polysomes (4,5). In our previous report (1), it was indicated that such functional differences might be explained by the difference of the distribution of messenger RNA (mRNA) and metabolic differences in mRNA were found between two species of liver polysomes (6). In an attempt to elucidate the difference in the species of mRNA on

free and bound polysomes, further efforts have been focused on the isolation of a specific kind of mRNA. As a first step, the isolation of serum albumin-synthesizing polysomes was attempted. The idea is that if polysomes are incubated with antiserum against serum albumin, nascent serum albumin on polysomes will react with the antiserum and only those polysomes which are synthesizing serum albumin can be isolated in the precipitate. Such an attempt was first made by Warren and Peters (7) with the use of $^{131}$I-labeled antiserum against serum albumin and recently similar procedures were employed by Higashi and Kudo (8) for detection of catalase synthesizing polysomes from rat liver.

## MATERIALS AND METHODS

Albino rats of Wistar strain weighing about 150-200g were used after overnight fasting in all the experiments. Total liver polysomes were prepared from the post-mitochondrial supernatant by the addition of sodium deoxycholate (DOC) at a final concentration of 1.3 %, followed by discontinuous sucrose density gradient centrifugation by the method of Wettstein et al (9) as described previously (1). Free and membrane-bound polysomes were obtained by the method shown in our previous paper (2). Each polysomal preparation was suspended in medium A' (0.25 M sucrose, 5 mM $MgCl_2$, 50 mM KCl, 10 mM $KHCO_3$, 50 mM Tris-HCl, pH 7.8).

Antiserum against rat serum albumin and ovalbumin were also prepared as described in our previous paper (3). To the polysomal suspension of the definite concentration, antiserum against serum albumin or ovalbumin was added. The mixture was kept for 80 min at 0°C and then the appropriate amount of serum albumin or ovalbumin, determined by the quantitative precipitin method of Heidelberger and Kendall (10), was added. After 60 min at 0°C, the solution was centrifuged at 3,000 rev./min for 10 min. The precipitate,

washed once with medium A' was dissolved in 1 N NaOH and the RNA and protein content were determined. The amount of polysomes co-precipitated with antigen-antiserum complex was expressed as a per cent of the total RNA of polysomes used in the reaction. In some cases, the supernatant after the immunological precipitation described above, was applied to sucrose density-gradient centrifugation to examine whether degradation of polysomes occurred during the incubation. For this purpose 0.2 ml of polysomal suspension before or after antigen-antiserum reaction was layered on the top of each 2.2 ml of 0.4 - 1.3 M sucrose gradient in medium A' which was layered over 0.5 ml of 2 M sucrose in medium A'. The centrifugation was carried out in a Hitachi RSP 40 A swinging rotor at 39,000 rev./min for 1 hr. Fractions of 10 drops each were collected, and each fraction was measured for absorbance at 260 mμ after the addition of 3 ml of water.

Partially purified ribonuclease inhibitor was prepared from the post-microsomal supernatant of rat liver by DEAE-cellulose column chromatography (11).

## RESULTS AND DISCUSSION

It is shown in Fig. 1 that the amount of polysomes in the precipitate depended on the ratio of the amounts of antigen and antiserum used in the reaction. RNase inhibitor was not used in this experiment. At the equivalence zone, a maximal amount of polysomes (about 3 % of polysomes used) was co-precipitated with the serum albumin - antibody complex. Since this value was not affected by the amount of polysomes used (Fig. 1-a and -b) and only about 0.7 % of polysomes was precipitated in the case of the ovalbumin - antibody reaction (Fig. 1-c), it was strongly suggested that those polysomes synthesizing serum albumin were specifically isolated by this method.

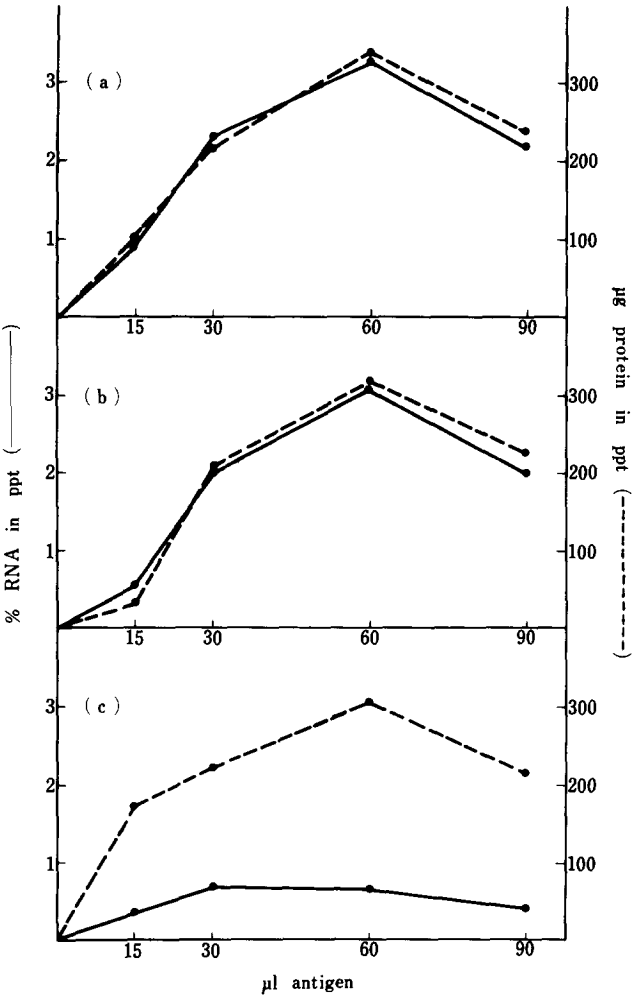


Fig. 1. Antigen - antiserum reaction in the presence of polysomes.
(a). Polysomes containing 150μg RNA and 40μl of antiserum against serum albumin were incubated in 1ml of medium A' for 80min at 0°C, then 15, 30, 60 or 90μl of 0.8mg/ml of serum albumin was added. After 60min, the precipitate was sedimented and washed once with medium A' and the RNA and protein content were determined.
(b). The same as (a), except that polysomes containing 300μg RNA were used in each reaction mixture.
(c). The same as (a), except that 80μl of antiserum against ovalbumin and 15, 30, 60 or 90μl of 0.1% ovalbumin were used.

Fig. 2 shows the sedimentation profiles of the supernatant fractions after removal of antigen - antiserum precipitates and of the original polysomes. When the reaction was carried out in

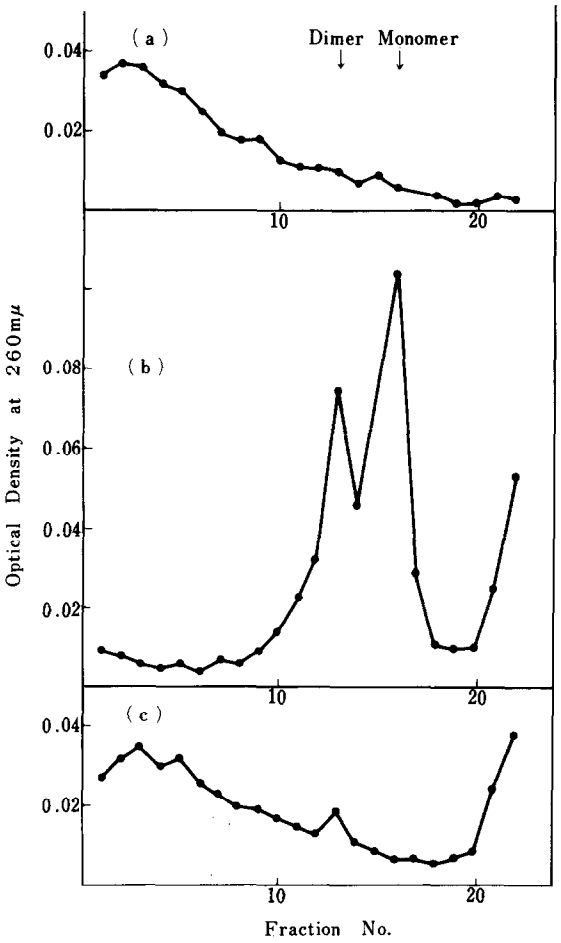


Fig. 2. Sedimentation profiles of polysomes.
Polysomes containing 170μg RNA were used in each reaction
(a). Without incubation.
(b). After incubation carried out as shown in Fig. 1-a using 60μl of serum albumin.
(c). The same as (b), but 50μl/ml of RNase inhibitor was present in the reaction mixture.
After incubation, each sample was centrifuged at 3,000 rev./min and 0.2ml of the supernatant was analyzed on sucrose density gradient centrifugation as described in the text.

medium A' without RNase inhibitor, most of the polysomes degraded into monomer and dimer (Fig. 2-b) and only about 3 % of polysomes used were found in the precipitate as in the experiment shown in Fig. 1. When the incubation was carried out in the presence of 50 μl/ml of RNase inhibitor, the degradation of polysomes was

almost completely prevented (Fig. 2-c). Furthermore, about 10 % of polysomes used were precipitated in the serum albumin - antibody reaction.

In the next experiment, free and membrane-bound polysomes were prepared from rat liver, and incubated separatedly with serum albumin and its antiserum or ovalbumin and its antiserum in the presence of RNase inhibitor. The result summarized in Table I clearly indicates that specific co-precipitation of polysomes with the serum albumin - antiserum complex occured only in the case of bound polysomes while a negligible amount of polysomes was found in the precipitate in the case of free polysomes. This result is in good agreement with that shown in our previous report (2), that is ; more than 10 % of nascent proteins released by EDTA from bound-polysomes of rat liver were immunologically characterized serum albumin, in contrast to the case of free polysomes in which only a negligible amount of serum albumin was shown in their nascent proteins. From these findings it may be concluded that by preventing the degradation of polysomes during the antigen - antiserum

Table I. Antigen - antiserum reaction in the presence of free or bound polysomes.
Free or bound polysomes containing 300µg RNA and 40µl of antiserum against serum albumin or 80µg of antiserum against ovalbumin were incubated in the presence of 50µl/ml of RNase inhibitor. Then 60µl of 0.8mg/ml of serum albumin or 60µl of 0.1% ovalbumin was added. The incubation was carried out as shown in Fig. 1.

| Antigen-antiserum | Polysomes | % RNA in precipitate |
|---|---|---|
| ovalbumin | free | 0.72 |
| serum albumin | free | 0.94 |
| ovalbumin | bound | 0.83 |
| serum albumin | bound | 11.8 |

reaction in the presence of RNase inhibitor, those polysomes synthesizing serum albumin can be very effectively isolated in the serum albumin - antiserum precipitate.

Some methods have been known for isolation of a specific kind of mRNA from mammalian cells. But their application is restricted to some cases in which the cells synthesize mainly one or a few kinds of the specific protein such as hemoglobin in reticulocytes, myosin in muscle and immunoglobulin in myeloma. The method for isolation of polysomes with a specific kind of nascent protein from a polysomal population with heterogeneous kinds of nascent proteins as described in this report, may be useful for the purification of a specific kind of mRNA from various kinds of cells. Isolation and characterization of mRNA of serum albumin from the immune precipitate described above are now in progress.

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