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The 3rd Quarterly REPORT ON

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SUBJECT OF INVESTIGATION

PHARMACOCHEMICAL STUDIES

ON

THE MICROSOMAL RIBONUCLEOPROTEIN

PARTICLES

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(6) PHYSICO-CHEMICAL STUDIES

ON

THE MICROSOMAL RIBONUCLEOPROTEIN PARTICLES.

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I. THE SCHEME OF THE INVESTIGATION IN THE THIRD QUARTER.

According to the research plan described in the second quarter report, the following experiments were carried out in this quarter.

1. The observation on the effect of Mg ion concentration on calf liver ribosome.

2. Experiments on the effect of EDTA on the ribosome.

3. Trials to separate the protein moiety of ribosome without serious denaturation and degradation.

II. RESULTS OBTAINED TO DATE

1. Mg ion concentration and calf liver RNP particles.

It has been recently reported (4, 6) that the microsomal RNP particles isolated from Escherichia coli were composed of 30s, 50s, 70s and 100s particles and the change of Mg ion concentration caused their reversible conversion to each other such as 100 s $\rightleftarrows$ 70 s $\rightleftarrows$ 50 s + 30 s. To standarize our ribosome samples, we have also made similar experiments on the effect of Mg^{++} ion concentration on the RNP particles isolated from calf liver.

When the ribosome was isolated by deoxycholate treatment as reported previously and suspended in Tris buffer (pH = 7.6, 1 mM Mg^{++}), it was mainly composed of 85 s particles with only a slight amount of 125 s and 65 s members. After 48 hr dialysis against the buffer of 0.25 mM Mg^{++} concentration, the ultracentrifugal pattern demonstrated its disintegration into 65 s and 45 s components, while the addition of $MgCl_2$ recovered the original 85 s particles reversibly. In the higher Mg^{++} concentration (5 mM) the RNP particles were converted to 125 s component and such a conversion was also reversible. It is apparent from these results that 125 s, 85 s, or 65 s - 45 s component of calf liver ribosome can be separated with relative ease by adjusting Mg^{++} concentration and we employed such a method in the following observations.

As described in the previous report, the 85 s component was observed electronmicroscopically as a nearly spherical particle of about 210 Å in diameter, but some of them showed the doublet character, a fact being consistent that of 85 s particles which consist of 65 s and 45 s particles. After removal of Mg ion, disintegrated RNP particles could be observed; rather more spherical and compact particles of somewhat smaller dimension were usually observed, while smaller, more asymmetrical particles appeared sporadically. The rather coarse background made it difficult to identify two or three sorts of particles, but probably the former would be 65 s member and the latter 45 s one. These findings are in fairly good agreement with those reported on the RNP particles of E. Coli (1, 3).

From these results, therefore, it could be concluded as in E. coli, that ribosomes in calf liver were also composed of four reversibly convertible components, only difference lying in their size.

2. Autodegradation of ribosomes and effect of EDTA.

It was already reported (4, 5) that the isolated RNP particles had ribonuclease (RNase) activity and the spontaneous breakdown of ribosomes on incubation at 37°C resulted from this activity. Recently Beer et al (1) reported that such a degradation was effected by removal of Mg^{++} due to addition of EDTA, about 90 % of the RNA being rendered acid-soluble. Hence the breakdown of each component of ribosomes in the presence or absence of EDTA was examined.

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After 2 hr incubation at 37°C, the ultracentrifugal analysis demonstrated the disappearance of 65 s and 49 s components, while 85 s or 125 s member remained nearly unaltered. Even in the former case, only a small part of RNA was rendered acid-soluble. Hence the lighter components seemed to be degradated only into a smaller subunit or oligonucleotides. When incubated in the presence of EDTA (0.02 M), however, all four components were broken down and considerable part of RNA was rendered acid-soluble. On the other hand, EDTA treatment of 85 s component at 0°C caused only its conversion to smaller components, only a small amount of acid-soluble RNA being recovered. At present, therefore, the RNase activity of the ribosome seems to be found only in its lighter components and latent in the heavier ones. Its apparent activation by EDTA would result from cleavage of heavier components to lighter ones due to removal of Mg^{++}. But we cannot yet exclude the posibility that EDTA has a direct action upon RNA molecule or upon its RNase activity.

From electronmicroscopical observations, EDTA treatment at 0°C appeared to render the 85 s particles somewhat loosened and flat, but spherical particles still remained. Some of them had tail-like fibrils attached to them. After incubation with EDTA at 37°, almost all spherical particles disappeared, flat, somewhat irregular ones being observed. Furthermore, many fibril-like structure as well as small granules could be observed. The flat structure seems to correspond to hollow cup-like structure described by Beer et al (1), while the fibrils appear to resemble the RNA molecules reported in the previous report. Because of considerably coarse background, however, we could not yet draw any conclusion on these respects. Further experiments will be required.

3. Experiments on the protein moiety of RNP.

Using electrophoretic, and ultracentrifugal method we tried to isolate the protein from RNP. But we failed to obtain protein samples enough to make physico-chemical or chemical measurements.

III. RESEARCH PLAN AT THE NEXT QUARTER.

1. Accomplishment of the experiments on the effect of EDTA on the RNP particles.

   Some observation stated above will be repeated to draw any conclusion on the stability and RNase activity of ribosomes.

2. Experiments on the protein moiety of RNP particles.

   This is the only one of the itemss left untouched which were included in this contract. Unexpected difficulties rendered all our trials unsuccessful. But further trials will be made.

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