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SUBJECT OF INVESTIGATION

PHYSICOCHEMICAL STUDIES
ON
THE MICROSOMAL RIBONUCLEOPROTEIN
PARTICLES

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⑥ PHYSICO-CHEMICAL STUDIES

ON

THE MICROSOMAL RIBONUCLEOPROTEIN PARTICLES .

~~Quarterly Progress~~ Quarterly Progress

⑨ Report. no. 2, 1-14 Sept 67

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I. THE SCHEME OF THE INVESTIGATION IN THE SECOND QUARTER

As stated in the previous quarterly report, the following items were studied.

1. Experiments on the physico-chemical and electron-microscopical studies on microsomal RNA were repeated.
2. The effect of heating on RNA.
3. Electron-microscopical observations of RNA particles.
4. In addition, preliminary experiments on the effect of EDTA on RNP particles as well as isolation of protein moiety from ribosome were attempted, but they were not yet successful.

II. RESULTS OBTAINED TO DATE

1. Physico-chemical properties and electron-microscopical observation of RNA molecules.

Some observations stated in the previous report (4) were repeated and the results reported hitherto (4, 5) could be confirmed.

2. The hyperchromicity of RNA due to raised temperature.

The samples of RNA of high molecular weight were prepared as stated previously (4).

When the temperature of RNA solution was raised from 10°C to 85°C for 15 minutes, its absorbancy at 258 m μ in 0.02 M phosphate buffer (pH=7.0) rose by 18-20 %. After cooling again to 10°C, the initial absorbancy was nearly completely recovered, a fact suggesting that such a hyperchromic effect of raised temperature is reversible. The ultracentrifugal sedimentation patterns were, however, irreversibly altered: only a slower, but remarkably homogeneous peak was obtained, its sedimentation constant being about 8 s. The sedimentation-viscosity molecular weight of this altered RNA was calculated in the same way as that of native RNA stated in the previous report (4, 5) and a value of about 10⁵ was obtained. Such a result is in fairly good agreement with that of Hall and Doty (2) and suggests that this component probably corresponds to their sub-unit RNA.

3. Electron-microscopical observation of heated RNA molecules.

Electron-microscopically, the effect of heating on the RNA molecules were also evidently observed; they were found to consist of shorter rod-like molecules, their length and width being 200-400 Å and about 20 Å respectively. But the more marked granular nature of the background in our pictures than in Hall's ones (1) made difficult to make an accurate measurement and comparison of finer structure smaller than 20 Å. Assuming the above-stated dimensions for this degraded RNA molecules, its molecular weight was obtained as the order of 10⁵, a fact which accords with ultracentrifugal analysis fairly well.

4. Electron-microscopical observation on ribosomes.

Employing the same electron-microscopical method as stated previously (1, 4), our samples of ribosomes were found to consist of round spherical

particles of considerable homogeneity. Their estimated average diameter and thickness were about 210 Å and about 160 Å respectively. When compared with the size estimated on thin-sectioned ribosomes, such a dimension of sprayed and shadow-cast ribosomes agreed fairly well with the former. By ultracentrifugal analysis, our ribosome samples were found to be mainly composed of 80-85 s component accompanied with 110-120 s. These particles would probably correspond to 80 s component.

When histogram of size distribution of ribosomes was constructed, however, we could prove the presence of particles whose length exceeds 300 Å (10 % or less). These particles were found as oval or diplococcal ones in the micrograph. Judging from their shape and size, it seems very likely that they are a dimer of spherical particles and correspond to 110 s component, (cf. Huxley and Zubay (3)). But there remains a possibility that they are an artifact formed by fusion or aggregation of two spherical particles during evaporation of microdroplet.

III. RESEARCH PLAN AT THE NEXT QUARTER

1. The observation of the effect of EDTA on the RNP particles.

Huxley and Zubay (3) reported already the dissociation of ribosomes into their subunit in the low Mg^{++} milieu. So the effect of EDTA on the ribosomes will be examined by electron-microscopical, ultracentrifugal and electrophoretic method.

2. Experiments on the protein moiety of RNP particles.

Preliminary experiments on separating native protein moiety from the RNP particles have been attempted but they were entirely unsuccessful. But further trials will be made. If it is not successful, drastic isolation method might be attempted.

IV. LIST OF REFERENCES

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