EVIDENCE FOR TWO PROTEIN FACTORS IN THE TRANSFER OF AMINO ACIDS FROM SOLUBLE-RNA TO RIBONUCLEOPROTEIN PARTICLES [1]

June M. Fessenden [2] and Kivie Moldave [3]

Department of Biochemistry, Tufts University School of Medicine
Boston, Massachusetts

Received October 24, 1961

Previous communications from this laboratory (1, 2) have described the preparation of a factor, probably an enzyme, from the soluble portion of rat liver homogenate that catalyzes transfer of amino acids from amino acyl-RNA to microsomal proteins. The present report describes the presence of an additional protein factor which is also required for transfer of the amino acyl group from soluble-RNA to the protein moiety of cytoplasmic ribonucleoprotein.

In experiments with intact microsomes it was observed that incorporation of labelled amino acids from soluble-RNA-amino acid-C^{14} into proteins required the presence of the non-particulate portion (100,000 X g supernatant) of the cell (1, 2). A highly purified soluble preparation (approximately 500-fold purified on the basis of protein content, as compared to the "pH 5-supernatant" fraction of homogenate supernatant) has been obtained (2). A similar requirement for a soluble fraction has also been reported from other laboratories and partial

---

[1] Supported in part by research grants from the American Cancer Society (P-177) and the U. S. Public Health Service (A-1397 and SF-264).

[2] Predoctoral Research Fellow of the U. S. Public Health Service.

[3] Senior Research Fellow of the U. S. Public Health Service.

purification of this transferring activity has been described (3, 4). Our recent findings that this factor is heat-labile under certain conditions, together with observations that it is non-dialyzable and salt-precipitable suggest that this activity is an enzyme. Ability to transfer a number of RNA-bound amino acids is increased to the same extent in the course of purification of this soluble factor (S-V), suggesting that this enzyme may catalyze transfer of several or perhaps all the soluble-RNA-bound amino acids to microsomal protein (2).

The present experiments have been carried out with purified ribonucleoprotein particles prepared from rat liver microsomes by extraction with deoxycholate essentially as described by Kirsch et al (5). Incorporation of radioactive amino acid from labelled amino acyl-RNA into purified ribonucleoprotein has been observed in the presence of crude ("pH 5-supernatant", S-II) or partially purified non-particulate fractions (2-4, 6); however, in contrast to incubations with intact microsomes, the purified soluble enzyme S-V failed to catalyze incorporation into ribonucleoprotein particles (Table I). As shown in Table I, amino acyl transfer, comparable to that observed with preparation S-II, occurs when the purified soluble factor S-V is supplemented with a preparation obtained from microsomal extracts (M-I). The microsomal preparation, a heat-labile, non-dialyzable factor, was obtained by extraction of rat liver microsomes with deoxycholate; these experiments were carried out with the crude dialyzed deoxycholate-soluble fraction which contained less than 4% RNA. Attempts to purify and characterize this fraction are in progress.

TABLE I

EFFECT OF SOLUBLE AND MICROSOMAL EXTRACTS ON AMINO ACYL TRANSFER

Additions	% C-^{14} Amino Acid Incorporated Into Protein
None	0
Crude soluble extract (S-II)	17
Purified soluble fraction (S-V)	0
Microsomal extract (M-I)	0
Purified soluble fraction (S-V) + microsomal extract (M-I)	24

Incubations contained buffered salt-sucrose media, soluble-RNA-leucine-C^{14} (0.1 mg. RNA, 1000 C.P.M.), GTP, and ATP as described previously (2), approximately 4 mg. of ribonucleoprotein, and 7 mg. of crude soluble extract ("pH 5-supernatant"), 0.007 mg. of purified soluble fraction (2), or 0.6 mg. of deoxycholate-soluble microsomal protein in a total volume of 1.9 ml. Time of incubation at 37°, 30 minutes. After incubation, the contents were diluted with media, centrifuged at 100,000 X g for 90 minutes to obtain the ribonucleoprotein, and RNA and protein were isolated as described (7).

In the absence of soluble fraction, this microsomal extract did not catalyze the transfer reaction.[4]

These results suggest the requirment for at least two separate activities in the transfer of amino acid from soluble-RNA to the protein component of ribonucleoprotein particles. One of the activities has been obtained from the soluble portion of rat liver homogenates and has been extensively purified; the other component has been obtained

[4] Incorporation of amino acids into the RNA moiety of microsomes and ribonucleoprotein particles, possibly intermediate in the incorporation of amino acids into protein, has also been observed and will be described separately.

from the microsomes but appears to be present in both the soluble and microsomal fractions. The finding that the crude soluble fraction S-II catalyzes the transfer reaction in the absence of microsomal extract M-I is consistent with this view; further, partial resolution of the two activities from homogenate supernatant has been obtained by salt-fractionation procedures. The microsomal factor is considerably more labile; its activity is rapidly lost even when stored frozen. The effects of glutathione on this system are variable and will be described in detail subsequently; although the combined enzyme preparations usually show a marked dependency on glutathione, it does not appear to replace either of the two factors described above and some preparations (for example, those described in Table I) have been obtained which are active in its absence.

REFERENCES

1. Grossi, L.G., and Moldave, K., Biochim. et Biophys. Acta, 35, 275 (1959).

2. Grossi, L.G., and Moldave, K., J. Biol. Chem., 235, 2370 (1960).

3. Takanami, M., and Okamoto, T., Biochim. et Biophys. Acta, 44, 379 (1960).

4. Von der Decken, A., and Hultin, T., Biochim. et Biophys. Acta, 45, 139 (1960).

5. Kirsch, J.F., Siekevitz, P., and Palade, G.E., J. Biol. Chem., 235, 1419 (1960).

6. Bishop, J.O., and Schweet, R.S., Biochim. et Biophys. Acta, 49, 235 (1961).

7. Moldave, K., J. Biol. Chem., 235, 2365 (1960).