THE BINDING OF AMINOACYL TRANSFERASE II TO RIBOSOMES

Lawrence Skogerson and Kivie Moldave

Department of Biochemistry

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

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Numerous studies indicate that aminoacyl transfer from aminoacyl sRNA to ribosome-bound peptidyl sRNA requires at least two protein factors (aminoacyl transferases I and II), GTP, Mg^{++}, a monovalent cation, and a reduced sulfhydryl compound (Grossi and Moldave, 1960; Bishop and Schweet, 1961; Fessenden and Moldave, 1963; Arlinghaus et al., 1963; Hardesty et al., 1963; Nathans et al., 1963; Nakamoto et al., 1963; Allende et al., 1964; Arlinghaus et al., 1964; Gasior and Moldave, 1965; Momose and Kaji, 1965; Lucas-Lenard and Lipmann, 1966). Studies with rat liver preparations (Sutter and Moldave, 1966) revealed that the initial rate of aminoacyl transfer was markedly stimulated when reduced transferase II was allowed to interact with ribosomes in the presence of GTP and NH_4^+ prior to the addition of transferase I and aminoacyl sRNA. Evidence is presented here that the effects of the interaction of transferase II with ribosomes may be due, in part, to the formation of a ribosome-transferase II complex that can be separated from free transferase II.

The biological preparations described below were prepared from rat liver. Aminoacyl sRNA containing ^{14}C-leucine and unlabled, esterified amino acids was prepared as described previously (Moldave, 1963); the specific activity was 140,000 cpm per mg RNA. Ribosomes, essentially free of contaminating transferase II were purified by centrifugation (22,500 rpm, Spinco No. 30 rotor, 24 hours) through a discontinuous

sucrose gradient containing 0.5 M NH_4Cl, 0.01 M Tris-HCl (pH 7.6) and 0.01 M $MgCl_2$; the top layer was 10 ml of 0.5 M sucrose and the bottom layer 10 ml of 1 M sucrose. Transferases I and II, more purified than reported previously (Gasior and Moldave, 1965) and completely resolved from each other, were prepared by modifications to be described in detail elsewhere (Schneir et al., in preparation); to obtain transferase I, the calcium phosphate gel eluate (Gasior and Moldave, 1965) was chromatographed on hydroxylapatite and eluted with 0.25 M potassium phosphate buffer (pH 6.8); for transferase II, the gel eluate was chromatographed on phosphocellulose and eluted with 0.01 M potassium phosphate buffer (pH 6.8) containing approximately 0.12 M potassium chloride.

Approximately 1.0 mg of ribosomes and 20 μg of transferase II were incubated in a total volume of 0.22 ml, with 1.5 μmoles of $MgCl_2$, 20 μmoles of NH_4Cl, and 15 μmoles of Tris-HCl (pH 8.0 at 0°), in the presence or absence of 0.05 μmole of GTP or 2 μmoles of dithiothreitol. After 5 minutes at 37°, the incubation mixtures were layered on discontinuous sucrose gradients containing 0.01 M $MgCl_2$ and 0.1 M Tris-HCl (pH 7.6); the top layer was 4 ml of 0.5 M sucrose and the bottom layer was 4 ml of 1 M sucrose; centrifugation was carried out at 38,000 rpm (Spinco, No. 40 rotor) for 4 hours. The sedimented pellets were resuspended in 0.40 ml of a solution containing 34 μmoles Tris-HCl (pH 8.0 at 0°), 3 μmoles $MgCl_2$, 40 μmoles NH_4Cl, and 1 μmole dithiothreitol. Such resuspended ribosomes were then incubated at 37° for 20 minutes with 20 μgm of transferase I, 20 μgm of ^{14}C-leucyl sRNA (2,800 cpm) and 0.1 μmole of GTP, in a total volume of 0.47 ml. At the end of the incubations, 0.5 ml of 10% trichloroacetic acid was added, and the suspensions were heated at 90° for 15 minutes. The acid-insoluble residues were collected on glass fiber filters and washed several times with 5% trichloracetic acid. The filters were then glued to planchets, dried and assayed for radioactivity in a low-background gas flow counter.

TABLE I

AMINOACYL-TRANSFERRING ACTIVITY OF RIBOSOMES SEDIMENTED FROM VARIOUS INCUBATION MIXTURES

Incubation Components[1]		C.P.M. Incorporated Into Protein
First Incubation	Second Incubation	
Complete	Complete	985
Complete, minus GTP	Complete	70
Complete, minus DTT	Complete	110
Complete	Complete, minus GTP	65
Complete	Complete, minus DTT	350

[1]The first incubation contained ribosomes, transferase II, buffered salts, GTP and dithiothreitol (DTT) in the amounts described in the text. The second incubation contained preincubated-sedimented ribosomes, transferase I, ^{14}C-leucyl sRNA, buffered salts, GTP and DTT in the amounts described in the text.

Table I shows some of the requirements for the binding of transferase II to ribosomes and for the subsequent reaction leading to polypeptide synthesis. When ribosomes, transferase II, tris buffer, $MgCl_2$, NH_4Cl, GTP and dithiothreitol were incubated, the ribosomes sedimented from such an incubation mixture catalyzed aminoacyl transfer in the absence of added transferase II (line 1); the level of incorporation is similar to that observed when fresh ribosomes are incubated directly in a complete aminoacyl transfer-reaction mixture or when transferase II-preincubated ribosomes are then incubated in the presence of additional transferase II. These results suggest that considerable amounts of transferase II are bound to the ribosomes during the first incubation. The binding of transferase II was markedly dependent on the presence of GTP (line 2) and dithiothreitol (line 3); when transferase II was absent from both the first and the second incubations, the level of incorporation resembled

that shown when either GTP or dithiothreitol was omitted from the first incubation. Ribosomes sedimented from incubations carried out in the absence of transferase II or GTP were fully active if these components were included in the second incubation.

Studies with ribosome-transferase II complex isolated by gradient centrifugation indicated that aminoacyl transfer was completely dependent on the presence of GTP (Table I, line 4) and partially dependent on dithiothreitol (line 5) during the polymerization phase of the reaction. Thus, GTP appears to be necessary not only for the binding of transferase II to ribosomes, but also in subsequent reactions leading to amino acid incorporation. This observation is consistent with the suggestions made previously (Sutter and Moldave, 1966) based on kinetic analysis with a similar system.

Preliminary experiments on the nucleotide specificity indicate that GDP and 5'-guanylyl-methylenediphosphonate (GDPCP) (Hershey and Monro, 1966) which are competitive inhibitors of the aminoacyl transfer reaction, are partially effective in the binding of transferase II to ribosomes. ATP is not active in this respect and it is possible that the apparent lower activity of GDP and GDPCP is due to the inhibitory effect of residual amounts of these nucleotides in the second incubation. In contrast to the highly specific role of GTP in peptide bond synthesis that may involve GTP hydrolysis and that is competitively inhibited by GDP and GDPCP, hydrolysis of GTP does not appear to be required for the binding of the enzyme to ribosomes. Th GTP effect at this step may be due to the interaction of GTP or its analogues at an allosteric of an active binding site.

The results presented here suggest that incubation of ribosomes and transferase II, in solutions containing GTP and sulfhydryl compound, leads to the formation of a product containing both ribosomes and transferase II. This complex, which can be separated from unbound transfer-

ase II by centrifugation, is active in the aminoacyl transfer reaction in the absence of added transferase II. Ribosomes isolated from mixtures incubated in the absence of transferase II, GTP, or sulfhydryl compound are fully active only when supplemented with transferase II. An additional role of GTP, related to this enzyme-ribosome interaction but not involving GTP hydrolysis, is suggested by these studies. Whether, in addition to the binding of transferase II to ribosomes, the preincubation reaction also leads to a ribosomal event priming ribosomes for the rapid acceptance of incoming aminoacyl sRNA, is under investigation.

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