THE BINDING OF AMINOACYL sRNA AND GTP TO TRANSFERASE I

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One of the protein factors (transferase I), required for the incorporation of amino acids from aminoacyl sRNA into rat liver ribosome-bound protein (Fessenden and Moldave, 1963; Gasior and Moldave, 1965), loses activity when incubated at 37° (Gasior et al., 1966; Ibuki et al., 1966; Ibuki and Moldave, 1967). Inactivation is more rapid in the presence than in the absence of GTP, and aminoacyl sRNA is the only component of the aminoacyl transfer reaction that protects the enzyme against loss of activity. When aminoacyl sRNA is incubated with transferase I, the rate of nonenzymatic deacylation is markedly lower than in the absence of the transfer factor (Ibuki et al., 1966). These observations suggested that the interaction of aminoacyl sRNA and transferase I yielded a product in which both components were more stable than either one in the free form. Evidence is presented in this report for the formation of a complex containing aminoacyl sRNA and transferase I activity, which can be separated from free aminoacyl sRNA by molecular sieve chromatography. The GTP-catalyzed loss of activity of transferase I is associated with a loss in the capacity of the enzyme to bind aminoacyl sRNA.

Purified ribosomes and transferase I and II (Skogerson and Moldave, 1967) were used. ^{14}C-leucyl sRNA (Moldave, 1963) had a specific radioactivity of 140,000 cpm per mg RNA and aminoacyl sRNA-^{14}C, extracted from E. coli (Wolfenden et al., 1964) grown in ^{14}C-uracil, had 10^6 cpm per mg RNA. The sRNAs were purified by gel filtration (Sutter and Moldave, 1966). Transferase I was incubated with aminoacyl sRNA-^{14}C and Tris-DTT-EDTA buffer (0.05 M Tris-HCl, pH 7.0, containing 1 mM dithiothreitol and 0.1 mM EDTA). The incubation mixture was then

applied to a column of Sephadex G-200 (1.5 X 25 cms), previously equilibrated with the Tris-DTT-EDTA buffer, and eluted with the same buffer. Aliquots from various eluate fractions were assayed for transferase I activity by incubation with the following components, in a total volume of 1 ml: 0.5 mg ribosomes, 80 mM Tris-HCl buffer (pH 8.0 at 0°), 6 mM $MgCl_2$, 80 mM NH_4Cl, 2 mM DTT, 0.2 mM GTP, 20 μg of ^{14}C-leucyl sRNA (2,800 cpm) and 26 μg of transferase II. This concentration of transferase II was saturating when assayed under these conditions. After 30 minutes at 37°, the hot (90°) trichloroacetic acid-insoluble fractions were prepared from the incubation mixtures, collected on glass fiber filters and the radioactivity determined in a low-background gas-flow counter. The results (transferase I activity) are expressed in the figures as cpm incorporated into protein X total volume in the eluate fraction / aliquot used in the assay. Various eluate fractions were also assayed for radioactivity from uracil-labeled aminoacyl sRNA-^{14}C. Aliquots were plated directly on planchets and radioactivity determinations were carried out with a gas-flow counter. In some cases, the cold trichloroacetic acid-insoluble material (aminoacyl sRNA) was precipitated using 1 mg of bovine serum albumin as carrier, collected on Millipore filters and counted. Both methods gave essentially similar results. The results are expressed as cpm of aminoacyl sRNA-^{14}C X total volume in the eluate fraction / aliquot counted.

Figure 1 shows the elution profiles of transferase I activity (open circles) and of aminoacyl sRNA (closed circles) from an incubation chromatographed on a Sephadex G-200 column. A considerable amount of ^{14}C from aminoacyl sRNA is associated with transferase I which emerges at the void volume, with a peak of activity at 14 ml (Figure 1 A). The rest of the aminoacyl sRNA-^{14}C is retarded by the column and emerges free of transferase I activity. When the concentration of transferase I is increased 10-fold (Figure 1 B), all of the aminoacyl sRNA emerged in the transferase I region. Thus, the binding of aminoacyl sRNA is related to the amount of enzyme.

GTP catalyzes the rapid and irreversible inactivation of transferase I

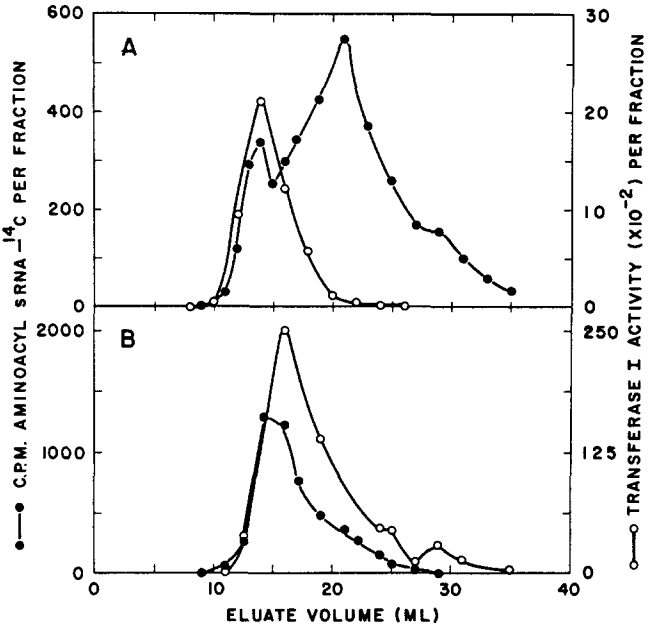

Figure 1. Gel filtration of transferase I - aminoacyl sRNA incubation mixtures. (A) Approximately 0.3 mg of transferase I and 10 μg of aminoacyl sRNA-^{14}C (10,000 cpm) were incubated with Tris-DTT-EDTA buffer, in a total volume of 1 ml. After 5 minutes at 23°, the reaction mixture was chromatographed on Sephadex G-200 and fractions analyzed as described in the text. (B) Approximately 3 mg of transferase I and 10 μg of aminoacyl sRNA-^{14}C were incubated, chromatographed and analyzed as described above and in the text.

(Ibuki and Moldave, 1967); the enzyme is protected against this nucleotide-stimulated loss of activity by aminoacyl sRNA but not by stripped sRNA or other polynucleotides. The binding of aminoacyl sRNA-^{14}C to GTP-inactivated transferase I was investigated. In this experiment, transferase I was incubated with GTP until approximately 15% of the initial activity remained. Aminoacyl sRNA-^{14}C was then added and after a few minutes, the reaction mixture was chromatographed on Sephadex G-200. Figure 2 reveals the markedly lower levels of transferase I activity that emerge from the column (open circles), and the distribution of aminoacyl sRNA-^{14}C (closed circles) which is no longer associated with the region where transferase I is usually eluted (eluate volumes of 12-20 ml). Thus, drastic inactivation of transferase I by GTP also leads to a loss in ability to bind

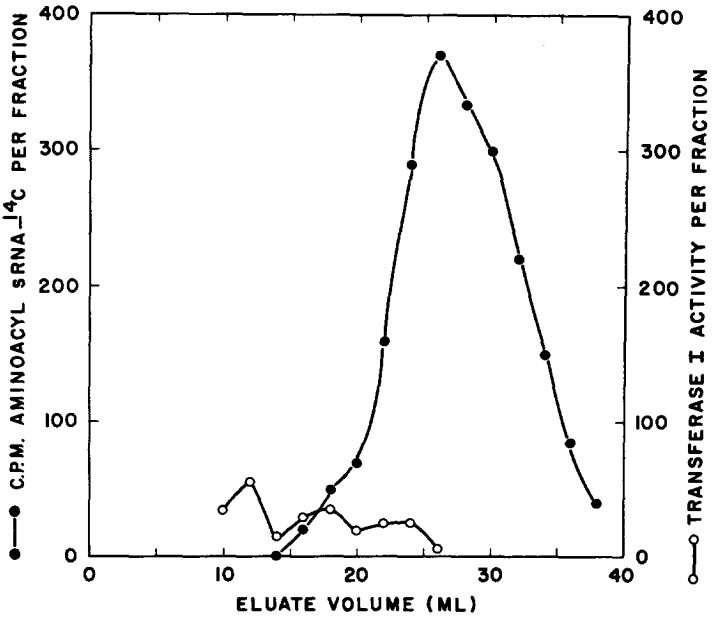

Figure 2. Gel filtration of GTP-inactivated transferase I-aminoacyl sRNA incubation mixture. Approximately 0.3 mg of transferase I and 2 mM GTP were incubated with Tris-DTT-EDTA buffer, in a total volume of 0.3 ml. After 5 hours at 37°, 10 µg of aminoacyl sRNA-^{14}C (10,000 cpm) were added and the incubation, at 23° in a total volume of 1 ml, was continued for 5 minutes. The reaction mixture was then chromatographed on Sephadex G-200 and fractions were analyzed for transferase I activity and ^{14}C as described in the text.

aminoacyl sRNA. When the GTP-dependent inactivation of transferase I was examined with time, it was found that the amount of aminoacyl sRNA bound was related to the amount of active enzyme remaining.

The binding of aminoacyl sRNA to transferase I appears to be a fast reaction, while the inactivation by GTP, after the first 20 minutes (Ibuki and Moldave, 1967) and particularly at high enzyme concentrations, is a relatively slow process; these observations suggest that GTP and aminoacyl sRNA may not compete for the same site on the enzyme. The observations made previously (Ibuki and Moldave, 1967) on the inactivation of transferase I by 5'guanylyl methylenediphosphonate, which is also protected by aminoacyl sRNA, further suggest that hydrolysis of GTP is not involved in this interaction. Whether these nucleo-

tides act at an allosteric site on the enzyme, influencing the binding of the substrate, remains to be determined. Similar experiments with radioactive GTP indicate that, concomitant with the loss of enzymatic activity, the nucleotide binds to transferase I. The binding of GTP, related to the interaction of transferase I with aminoacyl sRNA (Ibuki and Moldave, 1967), and the roles of transferase I and GTP in the enzymatic binding of aminoacyl sRNA to ribosomes, will be described subsequently. Binding of GTP to transfer factors has also been observed with reticulocyte (Hardesty et al., 1967) and E. coli (Allende et al., 1967) preparations.

Association of stripped sRNA with transferase I has also been observed; however, the results presented above, and the observation that stripped sRNA does not prevent the loss of activity of transferase I (Ibuki et al., 1966), are consistent with the suggestion that the interaction of aminoacyl sRNA and transferase I plays a role in the aminoacyl transfer reaction.

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