# INTERACTION OF TRANSFERASE II WITH THE 60 S RIBOSOMAL SUBUNIT

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## 1. Introduction

Elongation of polypeptide chains in mammalian systems requires two soluble protein factors, transferases I and II. Transferase I has been shown to function in the binding of aminoacyl-tRNA to ribosomes [1, 2], and evidence has been reported that transferase I (or a complex containing it) interacts specifically with the 40 S ribosomal subunit [3]. Transferase II has been demonstrated to function in translocation of peptidyl-tRNA from the acceptor to the donor site on ribosomes [2, 4, 5], but the exact location of its action is not known. Here we report evidence that transferase II from rabbit reticulocytes binds specifically to the 60 S ribosomal subunit. These results correlate with reports of a specific interaction of the translocation factor from *E. coli* (G factor) with the larger (50 S) ribosomal subunit from that organism [6, 7]. Data are also presented on the distribution of transferase II in the supernatant and ribosomal fraction of reticulocytes.

## 2. Methods

Transferase II, either free or ribosome-bound, was assayed by measuring transfer of the adenosine diphosphate ribosome moiety (ADPR) of $NAD^+$ into covalent linkage with the transferase [8], a reaction catalyzed by a fragment of diphtheria toxin [9, 10]:

$$\text{transferase II} + NAD^+ \rightleftharpoons \text{ADPR-transferase II} + \text{nicotinamide} + H^+$$

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The equilibrium of the reaction is far to the right at neutral pH or above [11]. Incorporation of label from $^{14}C$-$NAD^+$, uniformly labelled in the adenosine moiety, into trichloroacetic acid (TCA) insoluble material was measured under conditions where the reaction was allowed to go to completion, and the quantity of transferase was then estimated on the basis of a single ADPR group being incorporated per molecule of transferase (E.S. Maxwell, personal communication). Each reaction mixture (0.25 ml) contained 40 mM tris-HCl, pH 7.6; 40 mM dithiothreitol; 4 mM EDTA; 44,000 enzyme units diphtheria toxin per ml (16 $\mu$g protein per ml); $^{14}C$-$NAD^+$ (420 $\mu$Ci/$\mu$mole); and up to 20 nM transferase II. The reaction was initiated by addition of the transferase, and after incubation for 20 min at 25°, was terminated by addition of 5 ml 5% TCA. The precipitate was collected on glass fiber filter discs, washed with TCA, and counted in a planchet counter.

Concentrations of EDTA in the range of 2–4 mM were found to be necessary for maximal incorporation of label into transferase II bound to ribosomes. Omission of EDTA markedly reduced both the rate and extent of reaction of ribosome-bound transferase, but did not affect reaction of free transferase. Substitution of magnesium acetate for EDTA markedly decreased the extent of the reaction. Transferase II bound to ribosomes is apparently protected from reaction with toxin and $NAD^+$ and becomes reactive only when released by disruption of ribosomes [11, 12]. Under the conditions described maximal incorporation of label occurred within 5 min with free transferase, but required about 15 min for ribosome-bound transferase. Incorporation of label was proportional to the amount of supernatant fraction or ribosomes present, and was not enhanced by increas-

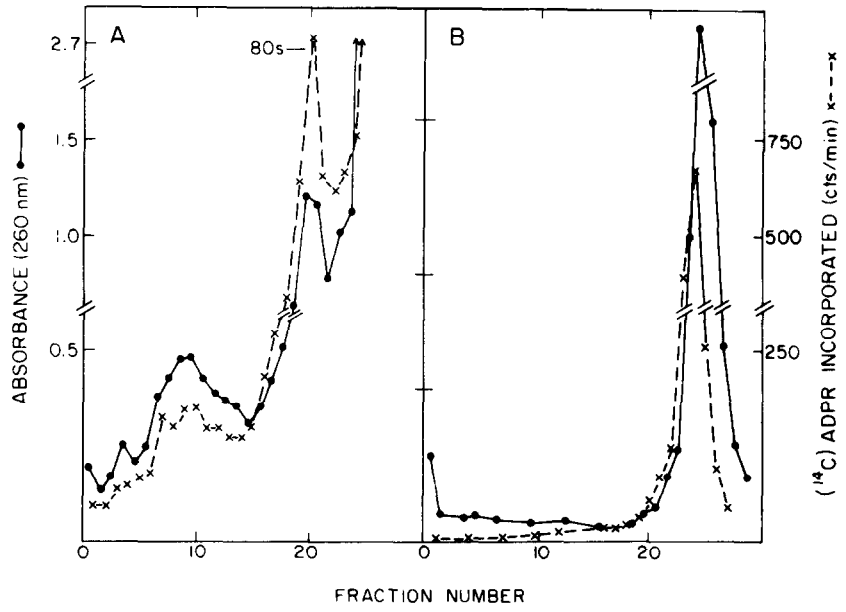


Fig. 1. Assay of transferase II in ribosome and supernatant fraction of reticulocyte extracts sedimented in sucrose gradients. (A) Whole cell extract (57 mg protein in 0.75 ml) was layered on a 12 ml sucrose gradient (15–40% w/v) in 10 mM tris-HCl, pH 7.6 containing 1 mM $MgCl_2$, and centrifuged for 2 hr at 35,000 rpm in a Spinco SW-41 rotor. The fractions were assayed for absorbance and transferase II as described under Methods. (B) Same as above, except that the protein concentration of the sample was ten-fold less, the gradient was 15–30% sucrose, and sedimentation was carried out for 9.5 hr at 41,000 rpm.

ing the concentration of toxin or $NAD^+$.

Methods of preparation of toxin, reticulocyte fractions, and $^{14}C$-$NAD^+$ employed have been described elsewhere [9, 13]. Endogenous $NAD^+$ was removed from reticulocyte fractions by passage through Sephadex G-25 equilibrated with 10 mM tris-HCl, pH 7.6; 15 mM KCl; 1.5 mM magnesium acetate.

## 3. Results and discussion

The concentration of transferase II in ribosomal and supernatant fractions isolated from rabbit reticulocytes by differential centrifugation was estimated in several experiments. The whole extract was found to contain about 1.5–2.0 molecules of transferase II per ribosome. Between 20 and 30 percent of the transferase was firmly bound to ribosomes, and could only be removed by treatment with high salt concentrations; the remaining 70–80 percent was in the supernatant fraction. This percentage of ribosome-bound transferase is similar to that reported for HeLa cells [14].

Fig. 1(A) shows results of an experiment in which reticulocyte extract which had been passed through Sephadex G-25 was sedimented in a sucrose gradient and the fractions containing various classes of ribosomes were assayed for transferase II. The peak of single ribosomes contained most of the ribosome-associated transferase, and in addition showed a higher specific activity of transferase II than the polysome fraction [15]. Using the value of 11.4 $A_{260}$ units per mg ribosomes, we have calculated that an average of about 0.3 transferase molecules per ribosome was present in the polysome fraction, while the single ribosomes contained 0.65 molecules transferase per ribosome. Fig. 1(B) shows that in a similar gradient centrifuged for a longer period free transferase II sedimented at about 5 S, just ahead of hemoglobin.

Several methods of dissociating ribosomes or polysomes into 60 S and 40 S 'derived' ribosomal subunits have been reported. Those which employ high concentrations of salt remove most of the transferase II from ribosomes. However, two other methods involving either treatment of ribosomes with 2.2 M urea in the presence of low concentrations of $Mg^{2+}$ [16],

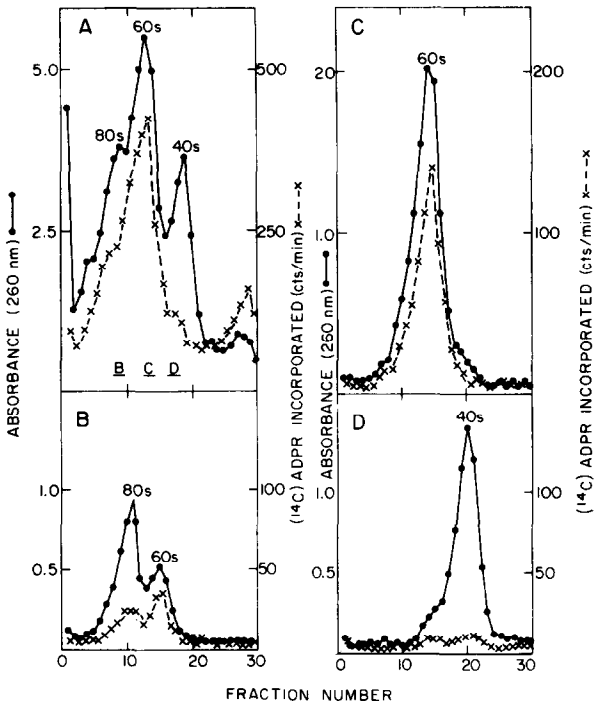


Fig. 2. Assay of transferase II bound to 80 S ribosomes and ribosomal subunits obtained by urea-treatment. Washed ribosomes (30 mg/ml in 1 mM potassium phosphate, pH 7.3; 30 mM KCl; 0.1 mM $MgCl_2$) were incubated in the presence of 2.2 M urea for 30 min at 4°, and then were dialyzed against buffer 1 (1 mM phosphate, pH 7.8, containing 10 mM KCl and 50 $\mu$M $MgCl_2$) to remove the urea. Samples of 0.75 ml were layered onto 12 ml, 5–20% (w/v) sucrose and centrifuged 2.5 hr at 41,000 rpm in a Spinco SW-41 rotor. Both gradients were fractionated and one was assayed for absorbance and transferase II (frame A). Fractions of the other from the 80 S, 60 S, and 40 S peaks were layered on individual 15–30% gradients and centrifuged 4.5 hr at 41,000 rpm, and the fractions were assayed as described. The material from the 80 S peak of the initial gradient is shown in frame B, that from the 60 S in frame C, and that from the 40 S in frame D.

or treatment with EDTA, dissociate the ribosomes producing little or no free transferase II.

Fig. 2a shows the distribution of transferase II among various fractions from a sucrose gradient of urea-treated ribosomes. The specific activity of transferase II was highest in the 60 S subunits and lowest in the 40 S subunits, the 80 S ribosomes exhibiting an intermediate value. To obtain better resolution of these species, fractions were isolated from the 80 S, 60 S, and 40 S regions of similar gradients and resedimented in individual 15–30% gradients before assay. The results (fig. 2b–d) verify those shown in fig. 2a and show that the 40 S subunit is virtually devoid of transferase II. The identities of the monosomes and 60 S and 40 S subunits were confirmed by measuring their sedimentation coefficients in the analytical ultracentrifuge.

A similar pattern of transferase was observed in gradients run on washed ribosomes treated with EDTA (2 $\mu$moles per mg ribosomes). The resulting ribosomal subunits sedimented at about 50 S and 29 S apparently due to unfolding of the structure [17]. Transferase II was associated with the larger subunit in a ratio of about 0.2 molecules transferase per particle, while the smaller subunit contained about 0.04 molecules transferase per particle.

Finally, when ribosomal subunits obtained by treatment of ribosomes with 1 M KCl (and containing less than one molecule transferase per 450 particles), were incubated with reticulocyte supernatant in the presence of 40 mM tris-HCl, pH 7.5 and 20 mM dithiothreitol most of the transferase bound to the 60 S subunit, only a trace being associated with the 40 S particles.

On the basis of these results we suggest that the action of the translocation factor in mammalian system may be localized on the larger ribosomal subunit, as has been reported for *E. coli* [6, 7]. Rao and Moldave [3] have studied binding of transferase II to ribosomal subunits from salt-washed ribosomes in a rat liver system, and have reported finding significant binding only in the presence of both ribosomal subunits. The differences in conditions of incubation and centrifugation may account for the difference observed.

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