Conformational change of 50 S ribosomes on enzymatic binding of phenylalanyl-tRNA

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Tight couple 70 S ribosmes are converted to loose couple ones on enzymatic binding of phenylalanyl-tRNA. Enzymatic binding at 0°C as well as nonenzymatic binding does not lead to any change. Further, no change takes place when the P site is occupied by *N*-acetylphenylalanyl-tRNA. Loose couple 70 S ribosomes are not affected by either enzymatic or nonenzymatic binding of phenylalany-tRNA.

70 S ribosome *Conformational change* *Phe-tRNA binding 50 S ribosome*

1. INTRODUCTION

That the ribosomal subunits undergo conformational change during protein synthesis has been widely accepted but to our knowledge no direct evidence is yet available. Lee et al. [1] observed the quenching of fluorescence of 50 S ribosomes with IAEDANS-labeled L7/L12, on enzymatic binding of Phe-tRNA and postulated the conformational change of 50 S ribosomes due to the binding. The conformational change of 50 S subunit on addition of EF-G and GTP, the agents responsible for translocation during protein synthesis, was demonstrated for the first time in this laboratory [2,3]. It has been shown that the loose couple 50 S ribosomes on treatment with EF-G, GTP and fusidic acid are almost completely converted to tight couples and the latter can be converted to loose ones (to the extent of 70%) on treatment with EF-G and $GMPPCH_2P$ or GMPPNHP in the presence of 30 S ribosomes and poly(U) [3]. Loose couple 70 S ribosomes are generally assumed to be damaged ones. Their conversion to tight couples led one of us to postulate that loose couple 50 S ribosomes are products of translocation and involved in protein synthesis [4]. It will be demonstrated here that tight couple 50 S ribosomes are also converted, to a large extent, to loose couples on enzymatic binding of Phe-tRNA in the presence of 30 S ribosomes and poly(U).

2. MATERIALS AND METHODS

Tight and loose couple 70 S ribosomes were prepared by ultracentrifugation at 4 mM Mg^{2+} as described [2]. The mixture of tRNA was prepared from the 100000 × *g* supernatant according to Zubay [5]. The mixture was acylated with [^{14}C]phenylalanine according to Scott [6] and its *N*-acetylation was done following the procedure of Haenni and Chapeville [7]. EF-T was prepared according to Gordon et al. [8]. Poly(U) was the product of Miles Laboratory, USA. Other reagents were of AnalaR quality.

2.1. *Enzymatic and nonenzymatic binding of phenylalanyl-tRNA*

The incubation mixture (0.5 ml) contained 0.05 M Tris-HCl, pH 7.6, 0.08 M KCl, 0.08 M NH_4Cl, 5 mM DTT, 0.5 nmol of tight or loose couple 70 S ribosomes, 1 nmol EF-T, 100 μg poly(U), 8 mM Mg^{2+}, 0.25 mM GTP and 1 nmol Phe-tRNA (10^5 cpm). In case of nonenzymatic binding EF-T and GTP were omitted and Mg^{2+} concentration was increased to 16 mM. In each

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case the incubation was carried out at 25°C for 25 min unless otherwise stated.

2.2. *Density gradient centrifugation*

An aliquot of each incubation mixture was dialysed against TMA (20 mM Tris-HCl, pH 7.5, 30 mM NH_4Cl, 6 mM β-mercaptoethanol and either 4 mM or 10 mM Mg^{2+}). The dialysed samples were applied on the top of 5–30% sucrose density gradients in the same buffer against which it was dialysed and subjected to centrifugation at 128000 × *g* in a Beckman L5-50 B ultracentrifuge for 2.5 h. The fractions (0.25 ml) were collected and counted in an LKB liquid scintillation counter. Absorbances were measured in a VSU II P spectrophotometer (Carl Zeiss, Jena).

3. RESULTS

It is well known that the poly(U)-dependent binding of aminoacyl-tRNA to 70 S ribosomes requires EF-Tu and GTP (review [9]). However, similar binding (nonenzymatic binding) takes place in the absence of EF-Tu and GTP in the presence of high Mg^{2+} concentrations. Tight couple 50 S ribosomes (along with 30 S ribosomes) were used for the binding of Phe-tRNA both enzymatically (at 8 mM Mg^{2+}) and nonenzymatically (at 16 mM Mg^{2+}). The extent of binding was checked by Millipore filter binding assay. Subsequently the incubation mixtures were subjected to sucrose gradient centrifugation in the presence of 4 mM Mg^{2+}. Prior to centrifugation the samples were dialysed against 4 mM Mg^{2+} and it was observed that no bound Phe-tRNA was released during the dialysis under this condition. As shown in fig.1, when Phe-tRNA was bound enzymatically about 70% of tight couple ribosomes were converted to loose couples (which do not associate at 4 mM Mg^{2+}). This has also been verified by kethoxal treatment [2]. Further, no change takes place if EF-T and GTP are added in the absence of Phe-tRNA as well as when Phe-tRNA is added in the presence of EF-T and $GMPPCH_2P$. Similarly, in the case of nonenzymatic binding there is no such change. The binding of uncharged tRNA also does not lead to any change (not shown). Similarly, if *N*-acetyl-Phe-tRNA is bound to tight couple ribosomes prior to the binding of Phe-tRNA tight couples remain unchanged (not shown) indicating thereby that if the P-site is occupied there is no conformational change. It should also be mentioned that when Phe-tRNA binds to loose couple 70 S ribosomes no such change is observed (not shown).

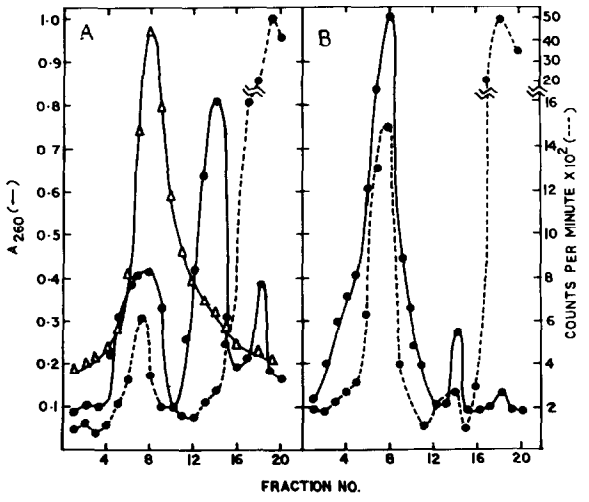

Fig.1. Effect of enzymatic binding of Phe-tRNA to 70 S ribosomes on the conformation of 50 S ribosomes. The incubation was carried out as described in section 2. In case of nonenzymatic binding EF-T and GTP were omitted and the incubation was carried out in the presence of 16 mM (instead of 8 mM) Mg^{2+}. The density gradient centrifugation of the incubation mixtures has also been described. (A) Enzymatic binding: (●) 4 mM, (△) 10 mM. (B) Nonenzymatic binding: (●) 4 mM. (——) A_{260}, (– – –) cpm.

It has been reported that tRNA remains bound at the A-site at 0°C and moves to the P-site (in case it is free) if the incubation is done at 37°C [10]. Therefore it was of interest to determine whether the conformational change of tight couple 50 S ribosomes takes place on enzymatic binding at 0°C. The binding was undoubtedly observed but there was no conversion whereas at 37°C the conversion does take place (fig.2). The most interesting situation is, however, observed on binding at 0°C and then increasing the temperature to 37°C. Similar (possibly somewhat less) extents of conversion were observed, as found earlier on incubation at 37°C. Similar results were obtained at 25°C as well. This shows that the simple enzymatic binding of Phe-tRNA is not capable of converting tight couples to loose couples but higher temperature is also necessary.

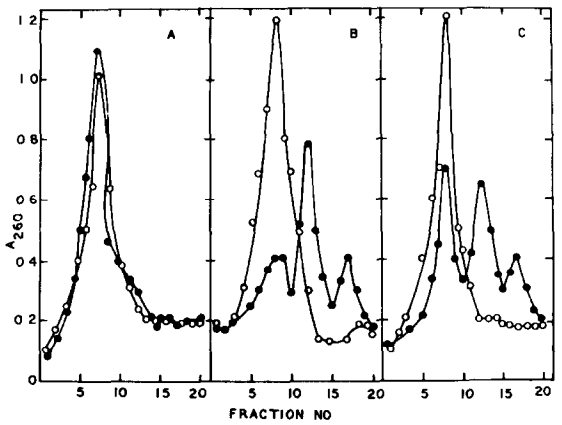

Fig.2. Effect of temperature on the conformational change of 50 S ribosomes due to the enzymatic binding of Phe-tRNA. The incubations were carried out as described in the legend to fig.1 except that the temperatures of incubations were 0°C (A) and 37°C (B). In case of (C) the incubation was carried out for 25 min at 0°C and subsequently the temperature was changed to 37°C and maintained for a further 25 min. All 3 mixtures were subjected to density gradient centrifugation as mentioned in the legend to fig.1. (A) 0°C, (B) 37°C, (C) 0°C⟶37°C; (●) 4 mM, (○) 10 mM.

4. DISCUSSION

The above-mentioned results clearly indicate that EF-Tu like EF-G is capable of inducing the conformational change of 50 S ribosomes (tight to loose couple conformation). Unlike EF-Tu, EF-G can carry out the conversion in both directions but is more efficient in inducing the change of 50 S ribosomes from loose to tight couple conformation. Further, EF-G (along with GTP) can induce the change at the 50 S ribosome level whereas EF-Tu can carry out efficient conversion at the 70 S ribosome level and in the presence of poly(U) and Phe-tRNA as well. The change induced by EF-Tu is effected only when the P-site is vacant. If *N*-acetyl-Phe-tRNA is bound earlier very little conversion takes place. Nonenzymatic binding also does not lead to any change. It should be noted here that it is known that the sliding of aminoacyl-tRNA from the A-site to the P-site is observed only when the P-site is free [10]. The temperature is also another important factor in such a change. At 0°C enzymatic binding takes place without any conformational change but the shift to higher temperature leads to the change.

It has been recently observed in this laboratory that when tight couple 50 S ribosomes are used in polyphenylalanine synthesis a small amount of loose couple 50 S ribosomes (10%) are formed (unpublished). It has also been shown that if the reaction is carried out in two steps, the first step (enzymatic Phe-tRNA binding) leads to the formation of about 70% loose couples (as recorded here) and the subsequent addition of EF-G and GTP results in the reconversion of most of the loose couple ribosomes to tight couple ones.

During protein synthesis the P-site is filled up first and then aminoacyl-tRNA occupies the A-site, therefore there is no scope of any conformational change being induced by EF-Tu under such conditions. The bindings of EF-Tu and EF-G are mutually exclusive (for discussion see [11]). The translocation induced by EF-G takes place only when EF-Tu leaves the 50 S ribosomes. GTP hydrolysis follows the binding of either one. This is necessary for the dissociation of both the factors from 50 S ribosomes (review [12]). No evidence has so far been obtained that the conformational change of 50 S ribosomes induced by EF-Tu during the translocation of Phe-tRNA from the A-site to the P-site has some physiological significance.

Since EF-G is approx. 2.5-times larger than EF-Tu and there are considerable homologies in the N-terminal sequences beween the two [13,14] it may be speculated that EF-G evolved at a later stage than EF-Tu. It is possible that the mechanism of initiation in protein synthesis also developed at a later stage. EF-Tu could have performed, in the early stage, the function of both aminoacyl-tRNA binding as well as translocation and still retains that capacity. At a later stage EF-G evolved to take up the latter job when the question of sophistication arose. Apparently, EF-Tu makes the ribosomes ready for translocation but the translocation does not take place as the P-site is occupied. EF-G at the next step completes the process initiated by EF-Tu.

Finally it should be pointed out that the change of 50 S ribosomes from the tight to loose couple conformation is due to the conformational change of 23 S RNA [2,3]. EF-Tu like EF-G is therefore expected to act at the 23 S RNA level.

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