J. Biosci., Vol. 11, Numbers 1–4, March 1987, pp. 561-569. © Printed in India.

Conformational change of L7/L12 stalk in the different functional states of 50S ribosomes

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Abstract. Conformational change of 50S ribosomes takes place during protein synthesis. The primary change is most likely in the secondary or tertiary structure of rRNA in the L7/L12 stalk region. In order to throw further light on this conformational change, the change in fluorescence of tight couple 50S ribosomes on conversion to loose couple 50S ribosomes containing 5-(iodoacetamido ethyl)-aminonaphthalene-l-sulphonic acid-labelled L7/L12, following the treatment with elongation factor-G and 5'-guanylyl methylene diphosphate was measured. It was enhanced in agreement with the results reported earlier. Further, the quenching of fluorescence of 50S ribosomes containing 5-(iodoacetamido ethyl)-aminonaphthalene-1-sulphonic acid-labelled L7/L12 by acrylamide was studied. The quenching is more in case of loose couples. On conversion of loose couple 50S ribosomes to tight couple ones the quenching becomes less whereas the reverse happens on conversion of tight couple 70S ribosomes to loose couples. These results indicate the conformational change of L7/L12 stalk in the different functional states of 50S ribosomes.

Keywords. Ribosomes; protein synthesis; conformation.

Introduction

It has been demonstrated for the first time in this laboratory that 50S ribosomes undergo conformational change during protein synthesis (Burma *et al.,* 1985a, b; Srivastava and Burma, 1985). Tight couple (TC) 50S ribosomes are converted to loose couple (LC) ones on binding of phetRNA^{phe} (Srivastava and Burma, 1985) and subsequently LCs are converted back to TCs during translocation (Burma *et al.,* 1986). It was shown earlier that TC and LC 50S ribosomes differ in the conformations of 23S RNA, most probably in the L7/L12 stalk region (Burma *et al.,* 1984). A new model of translocation was proposed on the basis of this (Burma, 1984; Burma *et al.,* 1985b).

L7/L12 stalk region which is comparatively mobile (Gudkov *et al.,* 1982; Tritton 1978; Van Diggelen *et al.,* 1971), is essential for many functions of ribosomes including translocation (Brot and Weissbach, 1981). In the original model proposed for translocation from this laboratory (Burma, 1984) L7/L12 stalk was arbitrarily put in extended and folded forms in TC and LC 50S ribosomes respectively Subsequently this was reversed (Burma *et al.,* 1985b) due to the report of Gudkov and Gongadze (1984) that the L7/LI2 proteins in 70S.elongation factor G (EF-G).5′-guanylyl methylene diphosphate (GMPP(CH_2)P) complex (ribosomes in the preGTP hydrolysis state) are digested by trypsin whereas in the 70S.EF-G.GDP.fusidic acid

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Abbreviations used: TC, Tight couple; LC, loose couple; EF-G, elongation factor G; GMPP(CH2)P, 5′-guanylyl methylene diphosphate; poly(U), polyuridynic acid; IAEDANS, 5-(iodoacetamido ethyl)-amino-naphthalene-1-sulphonic acid; GMPP(NH)P, 5'-guanylyl imidodiphosphate; EG-Tu, elongation factor Tu.

complex (ribosomes in the post GTP hydrolysis state) the L7/L12 proteins are trypsin resistant. This is also in agreement with the crosslinking data of Traut *et al.* (1983). It will be demonstrated in the present communication that the positions of L7/L12 in tight and loose couple 50S ribosomes are directly related to the functional states of 50S ribosomes.

Materials and methods

Materials

GTP and fusidic acid were obtained from Sigma Chemical Company, St. Louis, Missouri, USA, polyuridynic acid (Poly(U)) was the product of Miles Laboratory, USA. 5-(iodoacetamido ethyl)-aminonaphthalene-l-sulphonic acid (IAEDANS) was the product of Molecular Probes, USA and obtained as gift from Dr. C. R. Cantor of the Department of Chemistry, Columbia University, New York, USA. Both 5'-guanylyl imidodiphosphate (GMPP(NH)P) and phetRNA^{Phe} were obtained from Boehringer Mannheim, GMBH, West Germany. Elongation factor-G (EF-G) and elongation factor Tu (EF-Tu) were prepared from S-100 fraction of the extract of *Escherichia, coli* MRE 600 according to the method of Gordon *et al.* (1971).

Methods

Preparation of TC and LC 50S ribosomes: TC and LC 50S ribosomes were prepared from 70S ribosomes by ultracentrifugation in the presence of 4 mM Mg^{2+} as described earlier (Burma *et al.,* 1985a).

Preparation of L7/L12 proteins and 70S and 50S core particles: L7/L12 proteins were extracted from 50S or 70S ribosomes in the presence of 1 M NH_4C1 and ethanol following the method of Hamel *et al.* (1972) and purified by passing through DEAE-cellulose column according to the method of Moller *et al.* (1972).

Preparation of IAEDANS-conjugate of L7/L12 proteins: L7/L12 preparation was dialysed against 50 mM sodium carbonate buffer, pH 9·4 containing 100 mM KCl (buffer A). Dialysed L7/L12 solution (1 mg/ml) was added to a weighed amount of IAEDANS, the molar ratio of dye to protein being 20. After thorough mixing the solution was kept in dark at 30°C for 2-3 h following which it was again extensively dialysed against buffer A. The complete removal of unattached dye was ensured by monitoring the dialysate by fluorescence measurement.

Reconstitution of L7/L12-deficient core particles and IAEDANS-conjugate of L7/L12 The core particles of 50S ribosomes depleted of L7/L12 were prepared as described above. The reconstitution was done at 37°C for 30 min in buffer B (10 mM Tris-HCl, pH 7·5, 10 mM magnesium acetate, 30 mM ammonium chloride and 6 mM *β*-mercaptoethanol). The molar ratio of L7/L12-conjugate to core particles was 5. After the incubation the reaction mixture was diluted with buffer B and the reconstituted

particles were collected by pelleting in high speed swinging bucket rotor of Beckman L5 Ultracentrifuge. The pelleting process was repeated to remove the unattached dye conjugate of proteins.

Fluorescence measurement: The fluorescence was measured in a total volume of 0·3 ml containing 10 mM Tris-HCl, pH 7·5, 25 mM magnesium acetate, 100 mM KCl, 6 mM β-mercaptoethanol and 1 A_{260} unit of 50S ribosomes or 1·5 A_{260} units of 70S ribosomes as described by Lee *et al.* (1981a, b) in a Perkin-Elmer LS-5 Luminescence spectrophotometer using quartz micro cell of 5 mm path length. The excitation was done at 360 nm and the emission spectrum was recorded in each case. When acrylamide (recrystallised from chloroform before use) was used as a quencher requisite amounts of 2 M acrylamide solution were added step by step to the contents of the cell (0·3 ml).

Conversion of LC to TC 50S ribosomes containing I AEDANS-labelled L7/L12: The procedure was the same as described by Burma *et al.* (1985a) for unlabelled ribosomes. LC 50S ribosomes (40 pmol) were treated at 37°C for 30 min with EF-G (200 pmol), GTP (2·5 nmol) and fusidic acid (5 mM) in a total volume of 0·2 ml of buffer C containing 50 mM Tris-HCl, pH 7·5, 160 mM NH_4C 1, 10 mM Mg^{2+} and 12 nM *β* -mercaptoethanol. Subsequently the mixture was cooled to 4°C and diluted to 0·3 ml with buffer C.

Conversion of TC to LC 50S ribosomes containing IAEDANS-labelled L7/L12: The conversion of TC 50S ribosomes to LC 50S ribosomes was carried out at the level of 70S ribosomes (Burma *et al.,* 1985a, 1986). In the first case the incubation was carried out at 37°C for 30 min in 0·2 ml of 50 mM Tris-HCl, pH 7·5 containing 160 mM NH_4 Cl, 10 mM Mg $^{2+}$ and 1 mM dithiothreitol having TC 50S ribosomes (40 pmol), equivalent amount of 30S ribosomes, EF-G (200 pmol), poly(U) (8 μg) and GMPP(NH)P (0·8 nmol). After cooling to 4°C the incubation mixture was diluted to 0·8 ml with the above-mentioned buffer. In the second case TC 50S ribosomes (40 pmol) along with equimolar amount of 30S ribosomes were treated at 25°C for 20 min with 8 μ*g* of poly(U), 80 pmol of EF-Tu, 0·25 mM GTP and 80 pmol of $phetRNA^{phe}$ in a total volume of 0·2 ml containing 50 mM Tris-HCl, pH 7·6, 80 mM KCl, 80 mM NH_4 CI, 5 mM dithiothreitol and 8 mM Mg^{2+}. After incubation this mixture was cooled to 4°C and diluted to 0·3 ml with the same buffer.

Results

Effect of interconversion of tight and loose couple 50S ribosomes on the fluorescence of 50S ribosomes containing IAEDANS-labelled L7/L12

It has already been demonstrated in this laboratory that the fluorescence of LC 50S ribosomes containing IAEDANS-labelled L7/L12 is enhanced on treatment with EF-G, GTP and fusidic acid (Burma *et al.,* 1986). However, on treatment of TC ribosomes with poly(U), $phetRNA^{Phe}$, EF-Tu and GTP the fluorescence was found to

be reduced. The former treatment leads to the coversion of TC to LC 50S ribosomes while the reverse happens in the latter case. These results can be rationalised if it is assumed that the L7/L12 stalk is in an extended form in LC 50S ribosomes and more towards the body of ribosomes in the tight couples. It has been shown earlier that TC ribosomes can be converted to LC ribosomes on treatment with EF-G and a GTP analogue, GMPP(CH_2)P (Burma *et al.,* 1985a). Therefore IAEDANS-labelled L7/L12 were incorporated into TC 50S ribosomes essentially following the method of Lee *et al.* (1981a, b). On treatment of TC 70S ribosomes containing IAEDANS labelled L7/L12, with EF-G and GMPP(NH)P the fluorescence becomes reduced (figure 1). It should be noted that the reduced value is somewhat more than that of LC 50S ribosomes. This is expected as the conversion is known to be approximately 50–60% (Burma *et al.,* 1985a). These results also support the earlier assumption that the L7/L12 stalk is in an extended form in LC 50S ribosomes and folded form in TC 50S ribosomes. This was found to be true by using acrylamide as a quencher as described below.

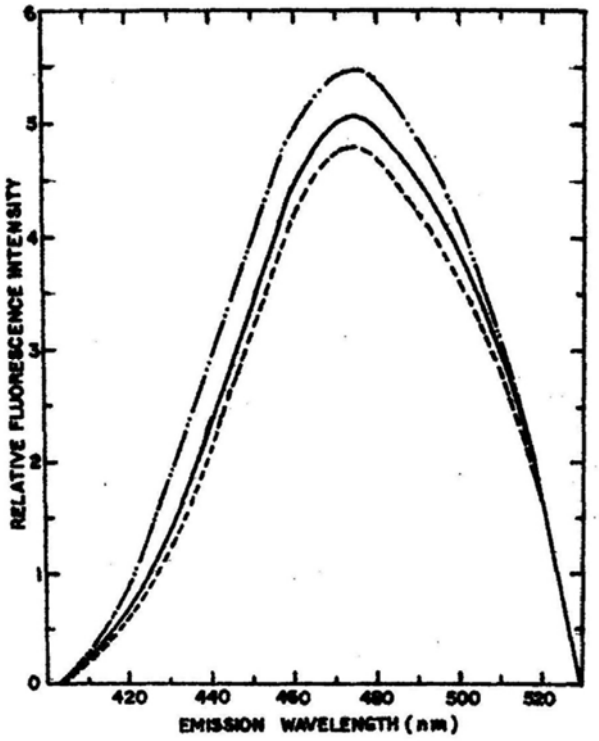

Figure 1. Quenching of fluorescence of 50S ribosomes containing IAEDANS-labelled L7/L12, on treatment with EF-G and GMPP(NH)P. The preparation of 50S ribosomes having IAEDANS-labelled L7/L12 has been described under 'materials and methods'. The method of measurement of fluorescence has also been described under 'materials and methods'.
(–··–), TC 70S ribosomes; (– – – –), LC 70S ribosomes; (—), TC 70S ribosomes treated with EF-G and GMPP(NH)P.

Quenching of fluorescence of 50S *and 70S TC and LC ribosomes containing IAEDANS-labelled L7/L12*

The quenching of fluorescence of TC and LC 50S and 70S ribosomes containing IAEDANS-labelled L7/L12 has been recorded in figure 2 as ratio of fluorescence at 475 nm in the presence and absence of acrylamide against the concentration of the quencher. The slopes of the curves indicate $K_q\tau_0$ values where K_q represents the association constant of the quencher with IAEDANS and τ_0 is the lifetime of fluorophor (in ns). The values turn out to be 7·27 (50S LC), 6·52 (50S TC), 6·85 (70S LC) and 6·00 (70S TC). These were fairly constant in a number of measurements. Unfortunately, it was not possible for us to measure τ_0 value but τ_0 is practically constant as it appears from the values reported by Lee *et al.* (198 1b). If it is so, it may be concluded that IAEDANS is more available for collision with acrylamide in 50S LC than TC ribosomes. As expected, the same is true in case of 50S ribosomes in comparison to 70S ribosomes. Therefore these data indicate that L7/L12 stalk is more exposed in LC than TC ribosomes.

Interconversion of tight and loose couple ribosomes and change of fluorescence of 50S ribosomes containing IAEDANS-labelled L7/L12

As mentioned already, LC 50S ribosomes are converted to TC 50S ribosomes on treatment with EF-G, GTP and fusidic acid whereas TC 50S ribosomes are con verted to LC 50S ribosomes on treatment with EF-G and a GTP analogue. (Burma *et al.,* 1985a). Further, similar conversion of TCs to LCs can be effected on treat ment with phetRNA^{phe}, EF-Tu and GTP in the presence of poly(U). The effects of such conversion on the quenching by acrylamide of the fluorescence of TC and LC 50S ribosomes containing IAEDANS-labelled L7/L12 are shown in table 1. $K_q\,\tau_0$ values are the slopes of the linear plot of F_o/F against $[Q]$, as discussed above and averages of 3 independent measurements (figure 2). When LC 50S ribosomes are converted to TC 50S ribosomes with EF-G, GTP and fusidic acid $K_q\ \tau_0$ value approaches from that of LC to TC (table 1). It is already known that the conversion is 90–95% under such condition (Burma *et al.,* 1985a). When TC 70S ribosomes were converted to LC 70S ribosomes in the presence of EF-G and GMPP(NH)P the value after conversion of TC to LC 50S ribosomes is much less. When TC 70S ribosomes are treated with phetRNA^{Phe} in the presence of poly(U), EF-Tu and GTP the fluorescence is 70% of the final products (table 1). Thus the quenching of fluoresce–nce corroborates the findings recorded earlier (Burma *et al.,* 1985a, 1986; Srivastava and Burma 1985).

Discussion

Ribosomes are known to exist in two different forms, tight and loose couples. TC ribosomal subunits (30S and 50S) do not dissociate at low Mg^{2+} concentration (4 mm or so) whereas LCs readily dissociate at this Mg^{2+} concentration. LC ribo–somes are much less biologically active and thought to be damaged ones (Noll *et al.,* 1973a, b; Hapke and Noll, 1976). It was shown for the first time in this laboratory that loose couples can be readily converted to biologically active tight couples on treatment with E F - G and G T P and more effectively in the presence of fusidic acid

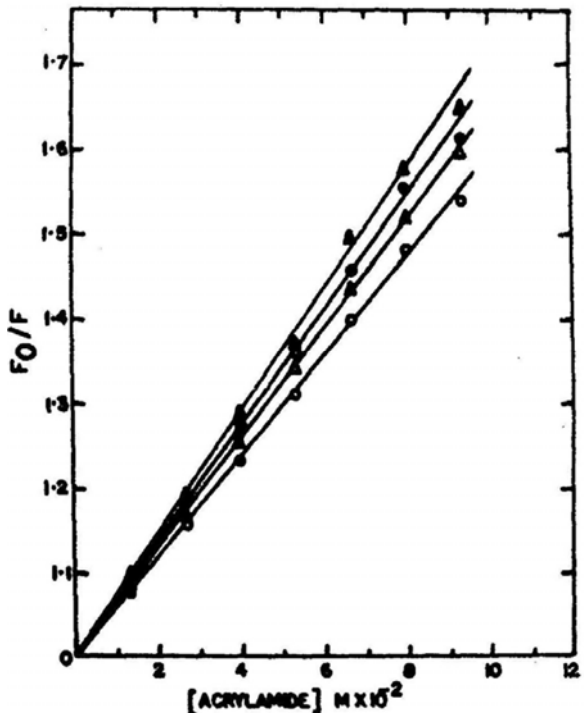

Figure 2. Quenching of fluorescence of IAEDANS-L7/L12-ribosomes by acrylamide. The method of measurement of fluorescence of 50S and 70S ribosomes having lAEDANS-labelIed L7/LI2 in presence *(F)* and absence *(F_o)* of acrylamide used as quencher has been described under 'materials and methods'. Straight lines have been drawn by the least square method.

(▲) LC 50S Ribosomes; (●) LC 70S ribosomes; (Δ) TC 50S ribosomes; (O) TC 70S ribosomes.

(Burma *et al.,* 1985a). Similarly TCs can be converted to LCs on treatment with EF-G and a GTP analogue (GMPP(NH)P or GMPP(CH_2)P). It has been further shown in this laboratory that TCs are converted to LCs on treatment with phetRNA^{phe}, EF-Tu and GTP (Srivastava and Burma, 1985). On the basis of the above-mentioned data it has been proposed (Burma, 1984; Burma *et al.,* 1985b) that LCs are not damaged ribosomes but actually 'intermediate' in protein synthesis. It has been further suggested that protein synthesis is initiated with TCs and during the binding of aminoacyl tRNA they are converted to LCs. However, during translocation of aminoacyl tRNA along with mRNA, LCs are converted back to TCs (Burma *et al.,* 1986). This cyclic process has been shown in figure 3.

It was first shown by Van Diggelen *et al.* (1971) that the differences between TC and LC ribosomes lie in the 50S subunits. It was unequivocally demonstrated in this laboratory that 50S ribosomes exist in two different conformations and that also due to two different conformations of 23S RNA (Burma *et al.,* 1984). Various studies also indicated that the major difference between TC and LC 50S ribosomes lies in rRNA in the L7/L12 stalk region. This is based on the observation that rRNA in TC 50S ribosomes in this region is more susceptible to the action of RNasel than that in LC 50S ribosomes. It was assumed that rRNA switches over from unfolded to folded

Table 1. Interconversion of light and loose couple ribosomes as indicated by the quenching of fluorescence of IAEDANS-L7/L12-ribosomes.

Ribosomal preparations	$K_q\tau_o$
LC 50S ribosomes	7·33
LC 50S ribosomes (treated with EF–G, GTP and fusidic acid)	6·66
TC 50S ribosomes	6·46
TC 70S ribosomes	6·00
TC 70S ribosomes (treated with EF–G and GMPP(NH)P)	6·44
LC 70S ribosomes	6·95
TC 70S ribosomes	6·00
TC 70S ribosomes (treated with EF–Tu and phetRNA^{phe} in presence of poly(U))	6·61
LC 70S ribosomes	6·94

The methods of treatment have been described under 'materials and methods'. The ratio of arbitrary fluorescence values in the presence and absence of acrylamide used as quencher was plotted against the concentration of the quencher as in figure 2. $K_q\ \tau_0$ values are the slopes of linear plots. The details have been described under 'materials and methods'.

conformation in this region, the switch over being controlled by EF-G and GTP (Burma, 1984; Burma *et al.,* 1985b, 1986).

L7/L12 proteins which occur in 4 copies, constituting the stalk region of 50S ribosomes, are known to be involved not only in translocation but also many other steps of protein synthesis (for review see Brot and Weissbach, 1981). These proteins are known to be mobile (Kischa *et al.,* 1971; Tritton, 1978; Gudkov *et al.,* 1982). In the model proposed from this laboratory for translocation in protein synthesis it has been assumed that L7/L12 proteins are responsible for the folding and unfolding of rRNA in this region (Burma, 1984; Burma *et al.,* 1985b, 1986). The assumption is strongly supported by the data presented in this paper.

Lee *et al.* (1981a, b) first demonstrated that there is quenching of fluorescence of L7-IAEDANS-70S ribosomes in the presence of poly(U) and phetRNA^{phe}. This indi cated the change in conformation of 50S ribosomes, specially in the L7/L12 stalk region. Similar fluorescence studies in this laboratory indicated the position of L7/L12 in more hydrophobic region in the TC 50S ribosomes than LC 50S ribo somes (Burma *et al.,* 1986). The present studies using acrylamide as quencher also support the above contention. These data indicate further that the L7/L12 stalk region is close to the main body of 50S ribosomes (representing TC 50S ribosomes) in the initial stage of protein synthesis. However, the alternate explanation of differential quenching due to differences in relative positions of L7/L12 dimers in the stalk region of 50S ribosomes cannot be ruled out. Work is in progress to understand the positions and involvement of L7/L12 during translocation. Anistropic measure ments carried out in this laboratory (unpublished results) have shown that L7/L12 stalk in 50S ribosomes is more mobile in tight than loose couples.

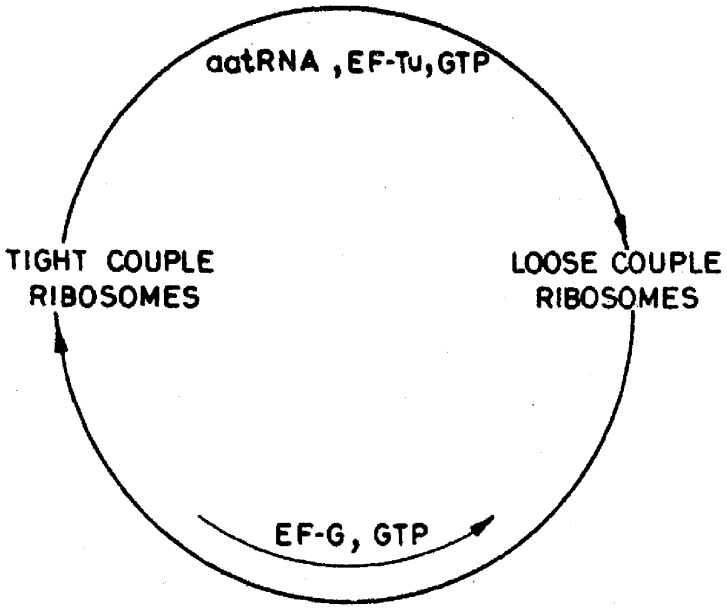

Figure 3. Cyclic process of conversion of tight and loose couple 50S ribosomes during protein synthesis.

Acknowledgements

The financial assistance was received from the University Grants Commission, Council' of Scientific and Industrial Research and the Department of Science and Technology, New Delhi. IAEDANS was a gift from Dr Charles Cantor, Columbia University, New York, USA. Thanks are also due to him for some valuable suggestions.

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