Movement of the 3'-end of 16 S RNA towards S21 during activation of 30 S ribosomal subunits

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Fluorescence techniques were used to study conformational changes that occur in inactive *E. coli* 30 S ribosomal subunits during activation by heating in 12 mM Mg^{2+}. Activation is associated with movement of a fluorophore on the 3'-end of 16 S RNA into a less polar environment and towards a probe on the cysteine thiol of ribosomal protein S21. The conformational change causes an apparent decrease in distance between the probes from 59 to 52 Å as determined by non-radiative energy transfer.

30 S subunit *Ribosome activation* *Protein S21* *3'-end 16 S RNA* *Fluorescence* *30 S conformation*

1. INTRODUCTION

Authors in [1] reported that 30 S subunits from *E. coli* ribosomes may exist in active and inactive interconvertible forms. Active subunits were inactivated by transient exposure to low Mg^{2+} concentration. Reactivation could be achieved by restoring the Mg^{2+} concentration to 10–20 mM and a brief heat treatment [2]. Active and inactive subunits differ in their ability to form 70 S ribosomes by association with 50 S subunits and to bind Phe–tRNA nonenzymatically. Several of the 30 S proteins, including S21, show a different reactivity to *N*-ethylmaleimide in the active and inactive states [3]. Authors in [4] reported that inactive 30 S subunits, in contrast to active subunits, cannot bind the deoxyoctanucleotide that is complementary to the 3'-terminus of 16 S RNA. They also found that active 30 S subunits missing protein S21 were unable to bind this oligonucleotide. They suggested that S21 was necessary to expose the 3'-terminus of 16 S RNA, probably by disrupting a segment of intramolecular base pairing between the 3'-end and a distal part of the 16 S RNA. It was further suggested [4] that in inactive 30 S subunits the same or a similar intramolecular interaction shielded the 3'-end of 16 S RNA. Psoralen crosslinking studies with inactive 30 S subunits provided direct evidence for an interaction of the 3'-terminal segment of 16 S RNA with 3 distant points of the RNA molecule in inactive subunits [5]. All these interactions appeared to be virtually eliminated in the activated subunit.

Here we report results indicating that inactivation and reactivation affect fluorescence from a fluorophore on the 3'-end of 16 S RNA and cause a change in the apparent distance to a fluorophore on the sulfhydryl group of S21.

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Abbreviations: CPM, 3-(4-maleimidylphenyl)-4-methyl-7-diethylaminocoumarin; FTS, fluorescein 5'-thiosemicarbazide; DCCH, 7-diethylaminocoumarin-3-carbohydrazide; 30 S(−S21), 30 S subunits from which protein S21 has been removed; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CPM-S21, S21 labeled at its sulfhydryl group with CPM; FTS-16 S RNA and DCCH-16 S RNA, 16 S RNA labeled at its 3'-end with FTS or DCCH

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2. MATERIALS AND METHODS

2.1. *Solutions*

Solutions were of the following compositions: (TMNSH solution) 10 mM Tris–HCl (pH 7.5), 10 mM $Mg(OAc)_2$, 100 mM NH_4Cl, 5 mM β-mercaptoethanol; (solution A) 20 mM Tris–HCl (pH 7.5), 10 mM $Mg(OAc)_2$, 1.5 M NH_4Cl, 1 mM dithioerythritol; (solution B) 30 mM Tris–HCl (pH 7.5), 20 mM $Mg(OAc)_2$, 500 mM KCl, 1 mM dithioerythritol; (solution C) 10 mM Tris–HCl (pH 7.5), 0.5 mM $Mg(OAc)_2$, 100 mM NH_4Cl, 5 mM β-mercaptoethanol.

2.2. *Preparation of labeled 30 S subunits*

Ribosomes and subunits were prepared as in [6] from a mutant of *E. coli* K12 strain A19, containing altered and more readily removable protein S21 [7].

Protein S21 isolated by the acetic acid–urea method [8] was a kind gift of Dr H.G. Wittmann (Max-Planck-Institut für Molekulare Genetik, Berlin). It was labeled under denaturing conditions by incubating for 30 min at 37°C with 1 mM CPM in 7 M guanidine–HCl, 10 mM Hepes–KOH (pH 7.5), followed by passage over a Sephadex G-25 column equilibrated with 7 M urea, 20 mM Hepes–KOH (pH 7.5), and finally dialysis against solution B. 16 S RNA was oxidized with periodate and labeled at its 3′-end with FTS or DCCH as previously described [6].

Incorporation of labeled S21 and/or labeled 16 S RNA into 30 S subunits was effected by the total reconstitution technique described in [6], except that total proteins extracted from 30 S(−S21) plus labeled S21 were used in place of total proteins from unmodified 30 S for incorporating labeled S21.

2.3. *Fluorescence measurements*

Fluorescence measurements were taken with an SLM photon counting spectrofluorimeter Model 8000, as in [9]. Steady-state fluorescence polarization and anisotropy measurements were made by using polarizers in the excitation and emission light paths.

2.4. *Inactivation and reactivation of labeled 30 S subunits*

Inactivation was essentially as described in [2] except that, instead of dialysis, stock solutions of labeled 30 S were diluted with an appropriate solution to give the final salt concentrations of solution C. This was then incubated at 28°C for 45 min. There was a progressive decrease in energy transfer during about the first 20 min of this incubation. Reactivation of inactivated 30 S subunits was achieved by raising the $Mg(OAc)_2$ concentration to 12 mM and incubating the sample for 15 min at 45°C.

3. RESULTS AND DISCUSSION

Reconstituted 30 S *E. coli* ribosomal subunits were inactivated by dilution to about 1.5 A_{260} units/ml in solution C, then reactivated by the addition of Mg^{2+} to 12 mM followed by incubation at 45°C for 15 min. The active and inactive ribosomes were analyzed for their ability to combine with 50 S subunits to form 70 S ribosomes by glycerol density gradient centrifugation. Without the incubation, less than 10% of the inactivated subunits could form 70 S ribosomes in TMNSH solution (not shown). After incubation at 45°C, about 80% of the 30 S subunits were in the peak of 70 S ribosomes. Comparable conditions for inactivation and activation of reconstituted 30 S subunits were used in the experiments described below.

The fluorescence properties of DCCH–16 S RNA free in solution or reconstituted into 30 S subunits were determined under conditions that cause subunit activation and inactivation (table 1). Fluorescence from coumarin is sensitive to the local environment of the probe and thus can provide a direct measure of changes in conformation near the fluorophore. Increasing Mg^{2+} from 0.5 to 12 mM causes an increase in the relative fluorescence from 1.00 to 1.50 with free 16 S RNA and from 1.38 to 1.59 with reconstituted 30 S subunits. Heating at 45°C with 12 mM Mg^{2+} causes a decrease in the relative fluorescence of both free 16 S RNA and 30 S subunits to about the same level, near 1.32. Anisotropy is near a value of 0.4 in all situations except for free 16 S RNA in 0.5 mM Mg^{2+}. In this case the emission maximum is at 485 nm but shifts to 481 or 480 nm in the presence of 12 mM Mg^{2+} and in all situations with 30 S subunits. The increase in the quantum yield and decrease in the emission maximum appear to

Table 1

Fluorescence properties of 3′-labeled[a] 16 S RNA free and in active and inactive 30 S subunits

Condition	Relative fluorescence	Anisotropy	Emission maximum
16 S RNA, 0.5 mM Mg^{2+}	1.00	0.34	485
16 S RNA, 12 mM Mg^{2+}	1.50	0.39	481
16 S RNA, 'activated'[b]	1.31	0.39	481
Inactive 30 S, 0.5 mM Mg^{2+}	1.38	0.39	481
Inactive 30 S, 12 mM Mg^{2+}	1.59	0.40	480
Active 30 S	1.33	0.39	480

[a] All 16 S RNA labeled at 3′-end with DCCH

[b] Heated at 45°C for 15 min in a solution containing 12 mM Mg^{2+} as described for activation of 30 S subunits

reflect a shift of the probe into a more hydrophobic environment. For comparison, the acetaldehyde derivative of DCCH has an emission maximum at 485 in the 12 mM Mg^{2+} solution and anisotropy of 0.26. In 95% ethanol the fluorescence intensity increases about 2.4-fold and the emission maximum decreases to 472 nm. Thus, considered together, the data of table 1 indicate that the 3′-probe on 16 S RNA is held quite rigidly in a somewhat hydrophobic environment, either free in solution or in 30 S subunits. The probe senses a change in conformation when either free 16 S RNA or inactive reconstituted 30 S subunits are heated in 12 mM Mg^{2+}. This change decreases the quantum yield in activated 30 S subunits to about the level for 30 S subunits in 0.5 mM Mg^{2+} and presumably involves the change in distance and/or relative probe orientation detected by

Table 2

Comparison of energy transfer between CPM–S21 and FTS–16 S RNA in active 30 S subunits and in 30 S subunits inactivated by exposure to low Mg^{2+} concentration

Condition of 30 S[a]	$[Mg^{2+}]$ (mM)	Quantum yield[b]	Energy transfer[c] (%)	r'[d]	Half-height limits of $Q(r'/r)$[d]	Limits of r[d]
Inactive	0.5	0.80	30	59	0.88–1.15	51–67
Inactive	12	0.78	39	55	0.88–1.15	48–62
Active, heated[e]	12	0.78	46	52	0.88–1.15	45–59

[a] Reconstitution, inactivation and reactivation procedures are described in section 2. Ribosomes were in solution C or solution C plus added Mg^{2+}, as indicated

[b] Quantum yield of the energy donor in the absence of the acceptor

[c] The observed percent energy transfer is calculated from the quenching of the donor fluorescence in the doubly labeled sample relative to the fluorescence of a sample of identical composition, except for having 30 S subunits reconstituted with unlabeled 16 S RNA. The value given has been corrected for the observation that only 80% of the ribosomes contain the fluorescein acceptor

[d] r' is the calculated distance between the probes. The distance at which energy transfer is 50% was calculated to be 51 Å assuming $\varkappa^2$, the probe orientation factor, is 2/3. r is the actual distance between the probes. The half-height limits of r were calculated according to the method in [10] using a polarization value of 0.45 for CPM, CPM–S21 and 0.33 for FTS–16 S RNA

[e] 30 S subunits were heated at 45°C for 15 min

energy transfer (table 2) and by other techniques, as described in section 1. The results of energy transfer measurements between CPM–S21 and FTS–16S RNA in active and inactive 30 S subunits are shown in table 2. Activation results in an increase in energy transfer and a decrease in the apparent distance between the sulfhydryl group of S21 and the 3′-end of 16 S RNA from 59 to 52 Å. Some increase in energy transfer is observed upon adding Mg^{2+} without heating. This appears to reflect an intermediate state in the activation process. After addition of Mg^{2+} at 28°C energy transfer increases over a 5 min period to a maximum of 39% (table 2, inactive 30 S in 12 mM Mg^{2+}).

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