E. COLI 30 S AND 50 S RIBOSOMAL SUBPARTICLE COMPONENTS IN THE LOCALIZATION REGION OF THE tRNA ACCEPTOR TERMINUS

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1. Introduction

Binding of aminoacyl-tRNA with ribosomal sub-particles seems to pass through two functionally important stages. At first the aminoacyl-tRNA binds with the 30 S-template complex by specific (codon–anticodon) and non-specific (for different aminoacyl-tRNA) interactions with the 30 S subparticle itself in the so-called A-site. On further addition of the 50 S subparticle to the obtained ternary complex at least the acceptor terminus of the aminoacyl-tRNA is located on the 50 S subparticle in the region of the peptidyl-transferase centre [1]. A number of experimental and logical grounds permitted us to suggest a model of the functioning ribosome in which the A-site and the peptidyl-transferase centre are situated on the surfaces of the 30 S and 50 S subparticles facing each other (see, e.g., [2,3]).

Little is known about the nature of this zone. The scarcity of information is probably explained at first by the attention of most researchers being focused on studying the role of just the protein component of the ribosome. The presence and role of ribosomal RNA are practically out of sight. The use of reagents specific only to certain amino acid functional groups (e.g., the –SH group [4–7]) for affinity labeling depletes the general picture of the studied zone as only those proteins are identified which, within the limits of mobility of the chosen reagent, contain a specific group accessible for its action.

We are convinced that this problem can be solved only by using absolutely non-specific affinity reagents capable of attacking any sterically close bond irrespective of its appurtenance to protein or to ribo-

somal RNA. Such reagents can be, for example, photoactivating compounds [8].

In the present study we have used N-acyl-[^{14}C]Phe-tRNA carrying photoreagent 2-nitro, 4-azidobenzoic acid (NAB) as N-acyl residue and we give the results of analysis of ribosomal subparticle components in the region of localization of the tRNA acceptor terminus.

2. Experimental

Preparations of *E. coli* MRE 600 ribosomes and [^{14}C]Phe-tRNA (specific activity of ^{14}C-phenyl-alanine is 500 mCi/mmole, Amersham) were obtained as described previously [9]. The 30 S subparticle preparation was kindly provided by Dr. N. V. Belitsina. N-Acylation of [^{14}C]Phe-tRNA by NAB N-hydroxy-succinimide ester was done as described by Lapidot [10] at pH 6.5. The degree of N-acylation determined according to Schofield and Zamecnik [11] was 90–100%. The conditions of NAB-[^{14}C]-Phe-tRNA binding with the ribosome were 20 min at 25°C in the dark. The content of the incubation mixture (1 ml) was: 4 mg of 70 S ribosomes (or 1.3 mg of 30 S subparticles), 1 mg of polyU, 0.5 mg NAB-[^{14}C]Phe-tRNA, 100 μmoles of KCl, 10 μmoles of HEPES, pH 7.2, and 20 moles of MgCl$_2$. Irradiation was carried out at 0°C for 3 hr in a thermostated quartz cell with a LETI emitter (USSR) (incandescent lamp, 400 W).

On completion of the photoreaction the mixture was precipitated with an equal volume of ethanol, the pellet was dissolved in the binding buffer but with 0.2 mM MgCl$_2$, and the tRNA not bound

covalently with the ribosome (or with the 30 S sub-
particle) was removed by centrifugation in the angle
rotor 28Y65 of the K-28 centrifuge (USSR) (4°C,
5 hr, 60000 rpm). The ribosomal pellet* was dis-
solved in a buffer containing 0.2 mM $MgCl_2$ and
centrifuged in a 5–20% sucrose gradient in a bucket
rotor 28C30 on the same centrifuge (4°C, 5 hr,
30000 rpm). Fractions containing the 50 S compo-
nent were mixed and after precipitation with ethanol
were dissolved in a buffer with 20 mM Tris–HCl,
pH 7.5 and 1 mM $MgCl_2$, to a 15–30 mg/ml con-
centration of ribosomes. Then an equal volume of
4 M LiCl + 8 M urea solution [12] was added to the
mixture and the ribosomal RNA was precipitated by
incubation at 0°C for 45–60 hr with a following
centrifugation (16000 rpm, 30 min on the K-24 cen-
trifuge, GDR). RNA was practically absent from the
supernatant. Distribution of the covalently bound
radioactive label between the ribosomal RNA and the
protein was determined by counting the radioactivity
of the pellet and supernatant in identical conditions
in the Triton X-100-toluene scintillator (1:2) on the
Model 3320 Packard TriCarb counter.

The isolated total 30 S protein was dialyzed
against water containing 0.1 mM mercaptoethanol.
The suspension was lyophilized, dissolved in an
electode buffer for electrophoresis [13] and treated
with pancreatic ribonuclease for hydrolysis of tRNA
and formation of corresponding protein-NAB-[^{14}C]-

* At photoreaction with 30 S subparticles the stage of
centrifugation in sucrose gradient was absent.

Phe-adenosine. Two-dimensional polyacrylamide gel
electrophoresis was done according to Kaltschmidt
and Wittmann [13]. Gel radioactivity was counted
in the above-mentioned scintillator after dissolving
the samples in a mixture of H_2O_2 + NH_3 [14] at
40°C for 24–48 hr. The radioactive spot was always
slightly shifted to the North-West relative to the spot
stained by Amido Black (cf. [15]).

3. Results

Table 1 gives the results of analysis of photo-
affinity-reagent binding specificity with the 70 S
ribosome. It is seen that in the presence of polyU up
to 90% of the introduced NAB-[^{14}C]Phe-tRNA is
contained in the ternary complex. In the absence of
polyU binding does not exceed 14%. Photoreaction
is activated by light (fig. 1) and proceeds in mild
conditions at sufficient effectivity: after 3 hr of
incubation at 0°C up to 20% of the NAB-[^{14}C]Phe-
tRNA bound into a complex remains covalently
fixed on the ribosome. The reaction is quite specific,
in the absence of polyU the effectivity corresponding-
ly drops according to the decrease of the degree of
binding (down to 5%, see table 1).

In complete agreement with the concept of
peptidyl-transferase centre localization, the reagent
attacks only the 50 S subparticle of the ribosome
(fig. 1).

The analysis of distribution of the label, covalently
bound with the 50 S subparticle, between the ribo-
somal protein and the RNA has shown that the label

Table 1
Specificity of binding and affinity labeling of ribosomes with NAB-[^{14}C]phenylalanyl-tRNA

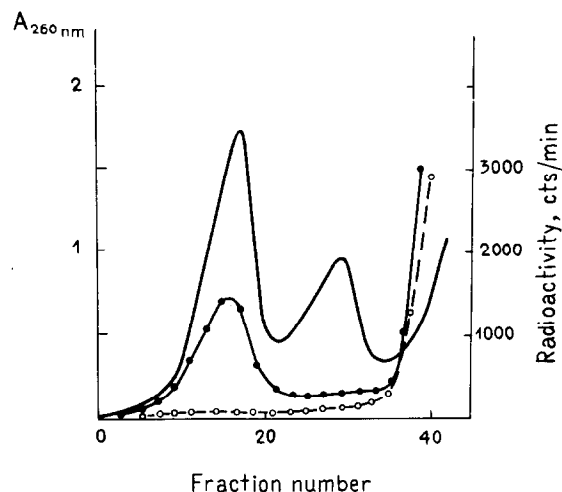| Ribosome	PolyU	Binding (%)	Affinity labeling			
			Temp. (°C)	Time (hr)	Effectivity (%)	RNA:Protein (%)
70 S	–	14	0	3	5	–
70 S	+	85–90	0	3	17–20	65:35
30 S	–	8–12	0	3	3–5	–
30 S	+	85–95	0	3	20–28	10:90
30 S	+	85–95	25	3	25	11:89

Fig. 1. Distribution of the radioactive label, covalently bound with the ribosome, between the subparticles after photoreaction with NAB-[14C]Phe-tRNA in the presence of polyU. (———) Optical density; (●———●) radioactivity; (o———o) radioactivity at incubation of the complex without irradiation (control).
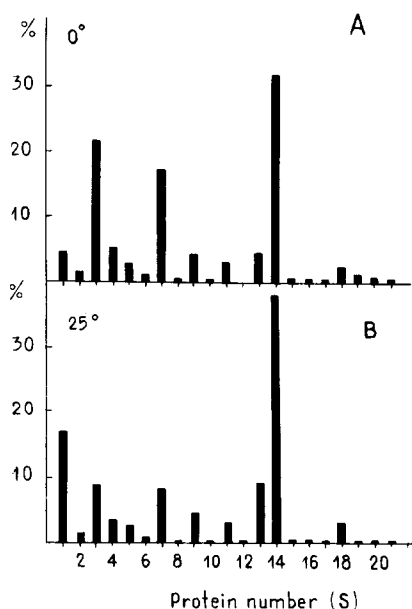

Fig. 2. Distribution of radioactivity between 30 S proteins on two-dimensional electrophoregram after photoreaction of NAB-[14C]Phe-tRNA with the 30 S subparticle in the presence of polyU at 0°C (A) and 25°C (B). Abscissa — the protein number according to the generally accepted nomenclature; ordinate — radioactivity in every protein in a per cent relation to the total radioactivity of the proteins.

is located mainly in the RNA (65–70%).

Within the ternary complex with the 30 S subparticle and polyU, the affinity reagent chiefly attacks the protein component when effectivity of the photoreaction is approximately 25% (see table 1). Data on identification of [14C]-labeled proteins by two-dimensional electrophoresis in polyacrylamide gel are given in fig. 2A. The main proteins reacting with NAB-[14C]Phe-tRNA within the specific ternary complex were S3, S7 and S14. Protein S13, the main one in the reaction N-bromacetyl-[14C]Phe-tRNA in an analogous complex (at 25°C) [7] proved to be a minor one together with some others (S1, S4, S9, S11). The rise of temperature of the photoreaction up to 25°C leads to an increase of the degree labeling of two minor proteins S1 and S13 at a simultaneous decrease of the label in proteins S3 and S7 (fig. 2B) without affecting the total effectivity of labeling of the 30 S subparticle and distribution of the label between the total 30 S protein and 16 S-RNA (see table 1).

4. Discussion

4.1. Exposure of the 50 S subparticle RNA in the region of the peptidyl-transferase centre

The ability of NAB-[14C]Phe-tRNA, within the specific ternary complex with the 70 S ribosome, and polyU to react mainly with the RNA component of the 50 S subparticle correlates with the data obtained by us earlier when using another non-specific affinity reagent, chlorambucilyl-[14C]Phe-tRNA [9]. At incubation of the latter within an analogous complex but in much more rigid conditions (15 hr, 20°C), up to 90% of the label covalently bound with the ribosome is found in the RNA of the 50 S subparticle. In the course of our studies data were published on affinity labeling of the peptidyl-transferase centre region of the E. coli ribosome by another photoactivated reagent, N-ethyl-2-diazomalonyl-[14C]Phe-tRNA [16]. The result obtained by the authors is analogous to ours: the attack proceeds mainly on the RNA of the 50 S subparticle.

Identical results obtained with the use of these non-specific affinity reagents with a different distance between the chemically active group and the acceptor terminus of tRNA lead to a conclusion that pre-

dominantly the RNA component of the 50 S sub-particle is most likely exposed in the region of the peptidyl-transferase centre near the acceptor terminus of tRNA. This result allows to raise the question on the possible role of this component in the functioning of the peptidyl-transferase centre. It is not excluded that the exposed oligonucleotide fragment of this RNA is involved in the interaction with the universal acceptor terminus of tRNA.

4.2. *Exposure of the 30 S subparticle proteins near the tRNA acceptor terminus*

In contrast to experiments with the 70 S ribosome, on irradiation of NAB-[^{14}C]Phe-tRNA, within the specific ternary complex with the 30 S subparticle and polyU, the label is chiefly located in the protein component. The main proteins forming the surface of the 30 S subparticle near the tRNA acceptor terminus were S3, S7 and S14.

The temperature rise of the photoreaction up to 25°C apparently leads to an increase of mobility of the tRNA acceptor terminus and/or a conformational change of the studied region of the 30 S subparticle surface. As a result of these changes, more remote or buried proteins, namely S1 and S13, become accessible for the action of the photoaffinity reagent. Hence it follows that under conditions for obtaining the specific ternary complex (25°C) besides proteins S3, S7 and S14, proteins S1 and S13 may be additionally exposed near the tRNA acceptor terminus.

Identification of the mentioned group of proteins (S3, S7, S14 and S1, S13) is of a certain interest for the functional topography of the ribosome. Thus, proteins S3 and S14 are included in the group of three proteins (+ S2) according to Randall-Haselbauer and Kurland [17] stimulating the enzymatic and non-enzymatic aminoacyl-tRNA binding with the 30 S-template complex and, as a consequence, participating according to the authors, in formation of the 30 S subparticle A-site. According to the data of Stöffler's group [18] protein S14 occurs at, or near, the ribosomal subparticle interface. Of the five proteins binding directly to 16 S-RNA, protein S7 is the only one situated in the 3' half of 16 S-RNA [19]. Proteins S3, S7, S14 and S13 are included in the group of proteins located at the 3'-terminal RNP-fragment obtained with a limited nuclease digestion of the 30 S subparticle [20]. According to Kenner's pre-

liminary data [21] protein S1 contacts directly with the 3' terminus of 16 S-RNA.

Correlation of our results with the presented facts allows us to conclude that within the specific 30 S-template complex the aminoacyl terminus of tRNA occurs near the ribosomal subparticle interface in the region of the 30 S subparticle A-site located close to the 3' terminus of 16 S-RNA. The conclusion on the A-site localization is especially interesting because it can help to understand the possible reason for the colicine E3 inactivating effect associated with splitting of the 50-nucleotide 3'-terminal fragment of the 16 S-RNA on protein biosynthesis [22]. This colicine E3-induced splitting may lead to a disruption of the conformation and function of the A-site, located according to our data in this region, and, as a consequence, to inhibition of aminoacyl-tRNA binding (cf. [23]).

Acknowledgements

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