SOME PROPERTIES OF DINITROFLUOROBENZENE-TREATED 50 S RIBOSOMAL SUBUNITS

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1. Introduction

It has been shown that the treatment of *E. coli* ribosomes (70 S particles) with DNFB*, an amino reagent which reacts only with ribosomal protein, had no effect on the capacity of ribosomes to bind poly U and on the reassociation of 30 S and 50 S subunits, whereas it prevented the poly U-coded binding of Phe-tRNA to ribosomes [1]. These observations suggest that ribosomal protein groups sensitive to DNFB are required for the later, but not for the former. In an attempt to elucidate the role of ribosomal proteins of 50 S subunits in the process of protein synthesis, this paper describes the effect of treatment of 50 S subunits with DNFB. It was found that the DNFB treatment of 50 S subunits completely abolished the capacity for polyphenylalanine formation, but DNP 50 S subunits retained their ability to form diphenylalanine to the same degree as the normal 50 S subunits.

2. Materials and methods

Ribosomes, their subunits (30 S and 50 S) and supernatant enzymes (S-150) were prepared from *E. coli* Q13 [2, 3]. Experiments for polyphenylalanine formation, diphenylalanine formation and binding of Phe-tRNA were done with the system as described previously [4, 5]. ^{14}C-Phe-tRNA was prepared according to Kaji et al. [6]. Poly U was purchased from the Miles Laboratories, and *E. coli* B tRNA from the Schwarz Bio Research Inc. ^{14}C-Phenylalanine (369 mCi/mmole) was obtained from the New England Nuclear Corp. (counting efficiency, 80%), and DNFB from the Tokyo Kasei Kogyo Co. Fusidic acid was kindly supplied by Dr. Akira Endo of the Sankyo Co.

DNP 50 S subunits were prepared following the procedure of Moore [1] with slightly modified conditions. Twelve mg of 50 S subunits (suspended in 2 ml of buffer A containing 0.01 M tris-HCl, pH 7.4; 0.01 M magnesium acetate; 0.06 M ammonium chloride; and 6 mM 2-mercaptoethanol) were layered on top of 28 ml of 10 to 30% linear sucrose gradient in 0.01 M cacodylate buffer, pH 7.0, containing 0.05 M KCl and 0.1 mM magnesium acetate, and centrifuged in a Spinco SW 25-1 rotor for 16 hr at 21,000 rpm at 4°. After the centrifugation, 50 S fraction was collected from the bottom of the tube and adjusted to a concentration of 0.01 M magnesium acetate. This suspension (3.5 mg of ribosomes/4 ml) was mixed with 0.05 ml of DNFB (10% in absolute ethanol), then maintained at about 27° with shaking for 4 hr, and dialyzed overnight versus buffer A at 4°. The DNP 50 S subunits were concentrated by the centrifugation at 65,000 rpm for 1.5 hr and stored in liquid nitrogen. The control 50 S subunits were treated in the same way as above with DNFB omitted from the reaction mixture

Abbreviations
DNFB : dinitrofluorobenzene;
DNP : dinitrophenyl-;
Phe-tRNA : phenylalanyl tRNA.

3. Results and discussion

In the preceding reports [4, 7], the ability of ribosomes to form polypeptide, to form dipeptide, to form a complex with aminoacyl tRNA has been found to be all sensitive to trypsin treatment of ribosomes in that order of decreasing sensitivity. These observations

Table 1
Effect of DNFB treatment of 50 S subunits on the polyphenylalanine and diphenylalanine formation and the binding of the Phe-tRNA.

	Subunits used			(A) Polyphenylalanine formed		(B) Diphenylalanine formed		(C) Phe-tRNA bound	
	30 S	50 S	DNP 50 S	(cpm)	(%)	(cpm)	(%)	(cpm)	(%)
1)	+	+	–	4855	100	3409	100	2532	100
2)	+	–	+	239	4.9	3437	101	1905	75
3)	–	+	–	83	1.7	116	3.4	54	2.1
4)	–		+	8	0.2	25	0.7	12	0.5
5)	+		–	128	2.6	77	2.3	1396	55
6)	* +		+			72			

(A) The reaction for polyphenylalanine formation was performed in three steps. The reaction mixture for the first step (formation of the 30 S subunit-poly U complex) contained the following in μmoles/0.08 ml: tris-HCl, pH 7.8, 10; magnesium acetate, 2; KCl, 5; 2-mercaptoethanol, 1.5. In addition, it contained 30 μg of 30 S subunits (preincubated for 5 min at 37°) and 30 μg of poly U. The mixture was incubated for 5 min at 37°. For the second step (reassociation of the above complex with 50 S or DNP 50 S subunits), 70 μg of 50 S or DNP 50 S subunits were added and incubated for 5 min. For the final step (polypeptide formation), to this mixture 0.08 ml of the polypeptide synthesis mixture containing the following in μmoles/0.08 ml was added: tris-HCl, pH 7.8, 5; magnesium acetate, 1; KCl, 2.5; 2-mercaptoethanol, 0.75; phosphoenolpyruvate, 1.6; GTP, 0.02. In addition, it contained 50 μg of S-150, 20 μg of pyruvate kinase and 20,000 cpm of ^{14}C-Phe-tRNA, and the reaction was allowed to continue for another 30 min. A 0.07 ml aliquot was assayed for the formed polyphenylalanine [8].

(B) The reaction for synthesis of diphenylalanine was carried out as described in table 1 (A), except that the reaction mixture contained 120 μg of 30 S subunits, 250 μg of 50 S or DNP 50 S subunits and 0.1 μmole of fusidic acid, and that the incubation was carried out at 24° in the final step. The total volume of reaction mixture was 0.12 ml and the pH value was 7.4. The formed diphenylalanine was analyzed by paper chromatography as described previously [4]. Under conditions where fusidic acid and excess of ribosomes were present, no appreciable amount of triphenylalanine and larger polymer was formed [4, 9, 10].

(C) The reaction for binding of Phe-tRNA was carried out as described previously [5], except that the total volume of reaction mixture was 0.05 ml and it contained 12 μg of 30 S subunits and 28 μg of 50 S or DNP 50 S subunits. A 0.05 ml aliquot was measured for the bound Phe-tRNA.

* Poly U was omitted from the reaction mixture.

suggest that the complexity of the reaction decreases in that order and that a different ribosomal protein is involved for each type of reaction required for protein synthesis. Accordingly, if 50 S subunits are pretreated with DNFB, one would expect that the ability of 50 S subunits to make polyphenylalanine should fall off effectively more than that of diphenylalanine formation. To investigate this possibility, experiments for the formation of polyphenylalanine and diphenylalanine were carried out in the presence of ^{14}C-Phe-tRNA and other components of polypeptide synthesis after the reassociation of DNP 50 S subunits with the 30 S subunit-poly U complex, and the resuls are given in table 1. The data shown in (A) of table 1 indicate that the treatment of 50 S subunits with DNFB severely reduced the capacity of 50 S subunits to form polyphenylalanine. About 95% of their capacity was abolished by the treatment.

In contrast, as shown in (B) of this table, the synthesis of diphenylalanine took place to the same extent as the control 50 S subunits. As shown by time course of diphenylalanine formation (fig. 1), a similar result was obtained even when S-150, GTP and other reagents were omitted from the reaction mixture. The amount of diphenylalanine formed by both systems increased in parallel up to at least 30 min. On the other hand, as shown in (C) of table 1, the DNFB treatment of 50 S subunits caused 25% loss of binding capacity for Phe-tRNA. In a separate experiment, when 30 S subunits were treated with DNFB, the binding of Phe-tRNA did not take place even in the presence of normal 50 S subunits, in agreement with the earlier work [1]. It has been reported that peptide bond formation is catalyzed by peptidyl transferase which is located in 50 S subunits and does not directly involve super-

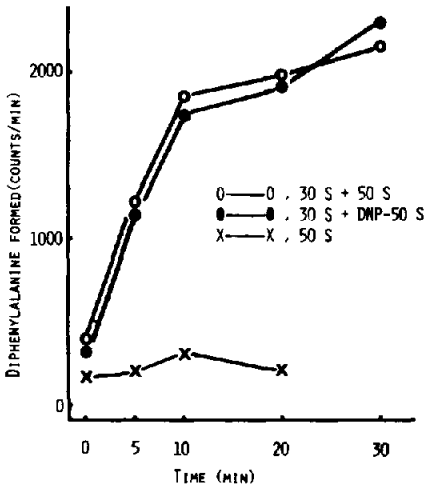

Fig. 1. Time course of diphenylalanine formation. The experiment was the same as in table 1 (B), except that 40 μmoles of ammonium chloride were added at the final step instead of S-150, GTP, phosphoenolpyruvate and pyruvate kinase. The final volume was 0.27 ml. Aliquots (0.05 ml) were taken at each time interval and the formed diphenylalanine was measured.

natant factors and GTP [9, 11, 12]. Peptide bond formation has also been shown to be facilitated on the presence of high monovalent cations (K^+, NH_4^+) [13, 14]. Although the partial loss of stimulatory effect by DNP 50 S subunits for Phe-tRNA binding has remained obscure, one can perhaps conclude from these data that DNFB treatment did not prevent the capacity of 50 S subunits to catalyze diphenylalanine formation, whereas it markedly impaired the ability to form polyphenylalanine. The DNFB treatment could, therefore, separate the dipeptide formation from more complex function of polypeptide synthesis. This seems to be consistent with the view that translocation differs significantly from the dipeptide synthesis with respect to trypsin sensitivity [4], requirement of factors [9], effect of antibiotics [15] and template size [16]. It is also reasonable to assume that the 50 S subunit structure would integrate some protein groups which involve movement of ribosomes along template, removal of deacylated tRNA and movement of peptidyl tRNA from A site to P site (translocation). These groups may be specifically sensitive to DNFB treatment. Additional evidences are, however, necessary to characterize the nature of the DNP 50 S subunits by measurements of their ability to split terminal phosphate of GTP (GTPase) [17, 18], to remove ribosome-bound tRNA [19–21], and to make aminoacyl puromycin [12, 13]. Further experiments are in progress along this line.

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