The stimulation of poly(U)-directed polyphenylalanine synthesis produced by modification of *Escherichia coli* ribosomes with *p*-hydroxymercuribenzoate, at low molar ratios of reagent to ribosomes, is due to an increase in the average chain length of polyphenylalanine synthesized, and not to the activation of inactive ribosomes. At a higher molar ratio of *p*-hydroxymercuribenzoate to ribosomes, which produces no overall change in activity, approximately 50% of the active ribosomes present in the untreated preparation have been completely inactivated, and the remaining active ones, like the ribosomes of the stimulated preparation, synthesize polyphenylalanine at an increased rate as compared with the untreated ribosomes.

Introduction

Although treatment of *Escherichia coli* ribosomes with pHMB or SucNBr at high molar ratios of reagent to ribosome inactivates their polypeptide synthesizing activity, at low molar ratios these reagents have a stimulatory effect that is produced by modification of the 50S subunit (1, 3). The stimulation produced by SucNBr is the result of an increase in the rate of polyphenylalanine synthesis by individual ribosomes, without activation of inactive ribosomes present in the preparation (3). The purpose of this work was to find out whether the stimulation obtained by modification with a classical sulfhydryl reagent like pHMB is also due to an increase in the rate of polyphenylalanine synthesis, and to determine the synthetic activity of the active ribosomes present in preparations partially inactivated by modification at higher molar ratios of pHMB to ribosomes.

Materials and methods

Ribosomes were obtained from *Escherichia coli* MRE 600 and washed with NH₄Cl as previously described (3). They were kept at -20°C in 5 mM Tris-HCl (pH 7.8), 20 mM magnesium acetate, 500 mM NH₄Cl, 2 mM dithiothreitol, 0.5 mM EDTA and 50% (v/v) glycerol, at a ribosomal concentration of 50-60 mg/ml. Ribosomal subunits were prepared by zonal centrifugation (2), and kept at -20°C in 10 mM Tris-HCl (pH 7.8), 10 mM magnesium acetate, 60 mM NH₄Cl, 2 mM dithiothreitol, 0.1 mM EDTA and 50% (v/v) glycerol, at a subunit concentration of 30-60 mg/ml.

Ribosomes and subunits were treated with pHMB in the absence of thiols. The ribosomal preparations were equilibrated with 10 mM Tris-HCl (pH 7.6), 10 mM magnesium acetate and 30 mM NH₄Cl by passage through a Sephadex G-25 column or by dialysis. The ribosomes and subunits thus obtained (5-7 mg/ml of ribosomes or subunits) were incubated with pHMB at 0-5°C for 16-18 h, and dialyzed against the above mentioned buffer overnight. Controls were subjected to exactly the same treatments as the modified preparations but in the absence of pHMB.

Polypeptide formation was assayed as poly(U)-directed polyphenylalanine synthesis in a crude system containing S-100 (100,000 x g supernatant) from which low-molecular-weight thiols had been eliminated by dialysis (3). The average length of the polyphenylalanine chains synthesized was obtained from the ratio of total phenylalanine incorporated to the phenylalanine incorporated in the amino terminal position.
The amount of amino terminal phenylalanine was determined by dinitrophenylation of the α-amino group of polyphenylalanine, hydrolysis and extraction of dinitrophenyl-phenylalanine with ether.

Results and discussion

The stimulation of polypeptide synthesis obtained by pHMB-modification of *Escherichia coli* ribosomes, like the stimulation by SucNBr treatment (3), is produced by an increase in the average chain length of polyphenylalanine synthesized, without activation of the inactive ribosomes present in the preparation (Fig. 1). At a higher concentration of pHMB (molar ratio of reagent to ribosome equal to 250), with an overall incorporation of phenylalanine equal to that in the untreated control, the polyphenylalanine chains synthesized had the size of those obtained with the stimulated ribosomal preparation, while the amount of active ribosomes decreased by 40%. These results indicate that about half of the stimulated ribosomes had been inactivated, but the active ribosomes left remained stimulated. At a molar ratio of reagent to ribosome equal to 500, the total incorporation decreased to 50% of the control, while the amount of active ribosomes was not changed, and the chain length of polyphenylalanine synthesized was about the same as in the untreated control, indicating that the additional modification has produced particles with the same activity as those untreated. The results obtained at the highest ratio of pHMB to ribosomes agree with those reported by Moore using the reagent N-ethylmaleimide (4).

Figure 2 shows that treatment with pHMB of 50S subunits stimulates polypeptide synthesis at low molar ratios of reagent to subunit, producing inactivation at higher concentrations of reagent, while treatment of 30S subunits is always accompanied by inactivation. Similar results have been reported earlier by Cronenberger and Erdmann (1) and in a study of the properties of SucNBr-modified ribosomes (3).

![Fig. 1. Chain lengths of polyphenylalanine synthesized by pHMB-modified ribosomes and proportion of active ribosomes. Ribosomes were treated with pHMB at the molar ratios of reagent to ribosome indicated. Polyphenylalanine synthesis, average chain length and active ribosomes were determined in aliquots of the pHMB-treated preparations and of the untreated control. They are expressed as percentages of the values obtained for the untreated control. The untreated control incorporated in 30 min 12.2 pmol phenylalanine/pmol ribosomes, with an average chain length of 115 phenylalanine residues, and 11% of active ribosomes.](image1)

![Fig. 2. Effects of pHMB-modification of 50S and 30S subunits on polyphenylalanine synthesis. 50S (●) and 30S (▲) subunits were treated with pHMB at the molar ratios of reagent to subunit indicated. Polyphenylalanine synthesis was determined in the presence of the complementary untreated subunit (ratio of untreated to treated subunit equal to 1.6). Activities are expressed as percentages of the activity obtained with the corresponding untreated control subunit. The incorporations in polyphenylalanine in 30 min were: 16.7 pmol phenylalanine/pmol 50S control subunit, and 4.7 pmol phenylalanine/pmol 30S control subunit.](image2)