DISSOCIATION OF PROTEINS FROM *ESCHERICHIA COLI* RIBOSOMES AFTER DIMETHYLMALEIC ANHYDRIDE TREATMENT

Effects of elongation factor G and antibiotics

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Received 15 September 1981; revision received 9 October 1981

1. Introduction

Modification of protein amino groups with dimethylmaleic anhydride (DMMA) causes the specific release of certain proteins from *Escherichia coli* ribosomes [1]. This phenomenon results from the formation of new electrostatic interactions caused by a net change of 2 charge units that accompanies the modification of an amino group [2]. The dissociation from the ribosomes of a particular protein as a result of DMMA modification will depend on:

(i) The number of amino groups modified in this protein;
(ii) The location of these groups within the protein, which will determine the extent of electrostatic interactions with other charged groups;
(iii) The interaction of the protein with neighboring ribosomal components.

The present paper describes how elongation factor G (EF-G) and the antibiotics viomycin, streptomycin and thiostrepton can affect the extent of dissociation of individual ribosomal proteins from *E. coli* ribosomes upon DMMA modification. EF-G is a supernatant factor that interacts with ribosomes during the translocation step in protein synthesis [3]. Viomycin, streptomycin and thiostrepton are antibiotics that block protein synthesis in prokaryotes [4]. Viomycin inhibits both initiation and elongation while streptomycin can prevent initiation, elongation and termination and can induce misreading. Thiostrepton interferes with ribosome reactions that involve EF-G binding and hydrolysis of GTP. We have found that EF-G and thiostrepton cause an overall protective effect against dissociation. These ligands lower the release by DMMA of proteins present at their ribosomal binding sites, presumably by steric hindrance of certain reactive amino groups normally available to DMMA. In contrast, both viomycin and streptomycin appear to induce changes in ribosome structure and exert their effects primarily by this mechanism rather than by a direct blocking of binding sites.

2. Materials and methods

Ribosomes from *Escherichia coli* MRE 600 were prepared as in [5]. EF-G, obtained according to [6], was a gift from Dr J. Modolell. DMMA was purchased from Sigma (St Louis).

Ribosomes (7.3 mg in 1 ml) were treated with DMMA, using a 16 000 molar excess of reagent relative to ribosomes, as in [1], in the presence and absence of 150 μM viomycin, 150 μM streptomycin, 15 μM thiostrepton, or 195 μg EF-G/ml. Experiments with either thiostrepton or EF-G involved incubation of these components with ribosomes for 10 min at 30°C prior to addition of DMMA. For EF-G, 29 μM guanyl-5′-yl methylene diphosphonate (Gpp(CH_2)p) was also included in the incubation mixture. After modification with DMMA, all preparations were centrifuged at 2°C and 63 000 rev./min for 2 h in a Beckman SW65 rotor. To regenerate modified amino groups, ribosomal 'core' particles were suspended in 1 ml of a solution containing 20 mM sodium cacodylate (adjusted to pH 6.0 with HCl), 20 mM magnesium acetate, 60 mM NH_4Cl and 0.5 mM EDTA, and incu-

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

Table 1
Effects of different ligands on the DMMA-induced dissociation of ribosomal proteins from ribosomes

Protein Spot	Protein present in the 'core' particles after DMMA-treatment (For each protein spot the percentage of incorporation shown is relative to 100% incorporation in the corresponding control)				
	Control in the absence of ligand	+Viomycin	+Streptomycin	+Thiostrepton	+EF-G
L1	52	66	60	63	108
L3	85	91	103	99	106
L5	108	71	101	100	102
L6	67	79	89	80	72
L7	75	75	64	110	100
L8	26	38	16	63	53
L9	87	93	78	110	101
L10	86	98	87	112	104
L11/S5	62	64	84	94	104
L12	73	76	62	113	99
L13	95	97	99	100	82
L16	93	87	98	101	84
L20/S2	105	101	106	97	105
L21	104	103	102	99	102
L25	78	64	73	101	105
L26	87	97	103	104	103
L27	103	61	82	85	98
L29	99	94	76	97	103
L32	95	79	82	97	104
L33	53	35	25	53	62
S1	7	18	6	14	53
S2	90	86	76	110	33
S3	92	68	103	79	82
S4	95	74	103	100	105
S7	86	81	103	92	104
S8	88	85	104	101	101
S9	96	93	90	79	99
S18	107	106	96	101	69
S19	99	98	92	100	103

The radioactivity incorporated in each sample (1 mg protein) was ~1 × 10^7 cpm, and levels for individual proteins were from 5000–175 000 cpm. The values obtained for different preparations were compared with a DMMA-untreated control by assuming that proteins L2, L4, L15 and S6 were always 100% present in the ribosomal particles. These proteins were never detected in supernatants, even when a large amount of protein was used in electrophoresis. Radioactivity values for protein spots were normalized with respect to values for spots L2 plus L15 (basic proteins) or L4 plus S6 (acidic proteins).

bated at 40°C for 45 min. Proteins were extracted from ribosomal 'core' particles with 67% (v/v) acetic acid [7] and the resulting solution dialyzed against 0.5% acetic acid, and finally lyophilized.

Extracted ribosomal 'core' proteins were labeled by reductive methylation with [^{14}C]formaldehyde [8]. Samples (1 mg protein) were dissolved in 0.5 ml 6 M urea and 0.1 M sodium borate (pH 9.0) and treated with 55 μl 75 mM [^{14}C]formaldehyde (20 mCi/mmol) at 0°C. After 4 min, four 20 μl aliquots of sodium borohydride (5 mg/ml) were added at 30 s intervals followed 1 min later by one 100 μl aliquot. The preparation obtained in this way was dialyzed against 0.5% acetic acid and lyophilized prior to electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was performed as in [9]. Gel slabs were stained with 0.04% Coomassie brilliant blue in 3.5% perchloric acid. Protein spots were excised from the gels, dispersed with a spatula, and extracted for 48 h at room temperature with 1.5 ml 70% (v/v) acetic acid containing 2.25 μg lysozyme. The samples were absorbed on glass-fiber filters which were then dried and assayed for radioactivity.

3. Results

The effects of viomycin, streptomycin, thiostrepton and EF-G on the release of certain individual ribosomal proteins as induced by DMMA are shown in table 1. After treatment of ribosomes with the reagent in the presence and absence of each ligand, the amounts of individual proteins in the 'core' particles were determined by measuring the radioactivity incorporated after reductive methylation of the proteins with [^{14}C]formaldehyde [8].

In the absence of ligands, treatment with DMMA allows dissociation from ribosomes of substantial amounts of certain proteins including L1, L6, L7, L8, L11/S5, L12, L33 and S1. Viomycin affects the release of proteins from both the large and the small subunits. At 150 μM, its presence during DMMA modification increases the extent of dissociation from ribosomes of proteins L5, L27, S3 and S4. Streptomycin (150 μM) increases dissociation of proteins L29 and L33, while decreasing that of proteins L6 and L11/S5. Both thiostrepton and EF-G produce an overall protective effect against dissociation. Of the total proteins listed in table 1, 10% more remain associated with ribosome particles after treatment with DMMA in the presence of either ligand relative to the effect of DMMA alone. More specifically, thiostrepton (15 μM) decreases the extent of dissociation from ribosomes of proteins L7, L11/S5, L12 and L25, and of the protein complex corresponding to spot L8. EF-G hinders dissociation of proteins L1, L7, L11/S5, L12, L25 and S1, and of the protein complex L8, while facilitating the release of proteins S2 and S18.

4. Discussion

Binding of a ligand to ribosomes can affect the DMMA-induced release of proteins in 2 possible ways. The ligand could sterically interfere with modification of the amino groups present at its ribosomal binding site. The resulting decrease in the number of modified amino groups at this ribosome location might lower the potential extent of protein dissociation. Alternatively a given ligand may affect protein dissociation indirectly by inducing a change in ribosome structure. Such a structural change could increase or decrease the number of amino groups accessible to DMMA, and could also affect the interactions of a particular protein with its adjoining components. Any direct action of a ligand on a particular ribosomal protein would be expected to protect it against release. In contrast, any indirect effect, by inducing changes in ribosome structure, could produce either an increase or a decrease in the dissociation of a given protein from ribosomes.

Viomycin affects the dissociation of ribosomal proteins from both 50 S and 30 S subunits, a result consistent with published data indicating that this antibiotic binds to each of the two subunits [10]. The increase in the extent of dissociation is likely to be a consequence of a change in ribosome structure rather than a direct blocking of the amino groups present at the viomycin binding sites.

Streptomycin produces an increase in the extent of dissociation from ribosomes of some proteins and a decrease for others, and in all cases 50 S subunit components are involved. Since streptomycin is known to bind to 30 S particles [4], the effects on dissociation observed here presumably result from changes in the structure of the 50 S particle that are induced by the binding of the antibiotic to its smaller counterpart.

Both thiostrepton and EF-G bind to 50 S ribosomal subunits, and their receptor sites overlap [11] since binding of the former ligand prevents binding of the latter [12]. The effects of thiostrepton and EF-G

on DMMA-induced protein dissociation are consistent with these facts. The 2 ligands protect against dissociation proteins L7, L12, L10 and L11, and some of these components are needed for EF-G-dependent GTP-hydrolysis [13].

Determination of the effects produced by binding of a ligand to a complex protein-containing structure on the DMMA-induced dissociation of individual ribosomal proteins provides an alternative procedure to study ligand binding sites and induced changes in the structure of ribosomal particles. Finally, it is also important to note that modification of ribosomes by DMMA, in the presence and absence of ligands, might provide a most valuable technique for the preparation of a family of 'core' particles with varying protein composition. This methodology could presumably be applied to other protein-containing structures such as chromatin and membranes.

Acknowledgements

We thank Dr Michael Cannon for critical reading of the manuscript. This work was supported in part by an institutional grant to the Centro de Biología Molecular from Fondo de Investigaciones Sanitarias (Spain).

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