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Protein-Deficient Ribosomal Particles from Yeast 60S Subunits Obtained by Modification with Dimethylmaleic Anhydride and by Treatment with NH_4Cl

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The protein-deficient particles prepared from yeast 60S subunits by modification of lysine residues with the reversible reagent dimethylmaleic anhydride are compared with those obtained by treatment with NH_4Cl. The two procedures cause selective dissociation of certain proteins. With a few exceptions, the dissociation pattern is similar in both cases. When using dimethylmaleic anhydride, a variation in the protein composition of the ribosomal cores is obtained by modification of the ribosomal subunits in the presence of any of the following ligands: elongation factor-2, ricin A, verrucarine A and puromycine.

Key words: Dimethylmaleic anhydride, 60S ribosomal subunits, Yeast ribosomes.

The complete dissociation of ribosomal particles obtained with monovalent cations plus urea indicates that the integrity of the ribosome is maintained by electrostatic interactions and hydrogen bonds (1, 8). Treatment with monovalent cations is the most widely-used procedure to obtain protein-deficient ribosomal particles from both prokaryotes and eukaryotes (1, 8). An alternative procedure, which makes use of the dissociation produced by modification of lysine residues with the reversible reagent dimethylmaleic anhydride (DMMA), has been employed for the partial reconstitution of yeast 60S subunits (14). The purpose of the present work is to compare the protein-deficient particles obtained from yeast 60S subunits by modification with DMMA to those prepared by treatment with NH_4Cl.

Materials and Methods

Materials. Ribosomal 60S subunits were prepared from *Saccharomyces cerevisiae* haploid strain Y166 (α, his4, trp5, MA1) as previously described (12). Elongation factor-2 was obtained from rabbit

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reticulocytes (4). DMMA (Sigma) was recrystallized from its solution in petroleum benzine boiling between 40 and 60°C.

Preparation of protein-deficient particles by treatment with DMMA. Ribosomal 60S subunits (1.5-3.0 μM) in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (K^+) (pH 8.2), 80 mM KCl, 12.5 mM $MgCl_2$ and 1 mM dithiothreitol (0.5-2.0 ml) were treated with the desired amount of DMMA, the pH being maintained at 8.2 by addition of base (14). To separate the ribosomal particles from the released proteins, the modified preparations were diluted with a suitable solution to 0.75-1.5 μM ribosomal particles and the following ionic conditions: 50 mM Tris-HCl (pH 7.4 at room temperature, approximately pH 8.0 at 2°C), 500 mM ammonium acetate, 100 mM $MgCl_2$ and 5 mM 2-mercaptoethanol. Centrifugation took place at 2°C and 30,000 rpm in a Beckman SW 50 rotor for 12-15 h. Centrifugation under these conditions has little effect on the activity of the untreated control 60S subunits. The pellet containing the ribosomal particles was suspended in 100-400 μl of 50 mM Tris-HCl (pH 7.4 at room temperature), 300 mM NH_4Cl, 15 mM $MgCl_2$, 250 mM sucrose, 15 % glycerol and 1 mM dithiothreitol. To regenerate the modified amino groups, the pH was lowered to a value close to 6.0 by dialysis against a buffer solution of this pH or by addition to the modified preparation of a low pH buffer, followed in this last case by incubation at 0-4°C for 24 h (7, 14).

Preparation of protein-deficient particles by treatment with NH_4Cl. The ribosomal subunits (1.0 μM) in 50 mM Tris-HCl (pH 7.4 at room temperature), 100 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and the chosen concentration of NH_4Cl were incubated at 37°C for 1 h. The ribosomal particles were separated from the released protein by centrifugation of the incubation mixture under the conditions used for the DMMA-treated preparations, and the pellet was suspended as indicated for these preparations.

Analytical procedures. Sedimentation was studied by centrifugation in linear 15-30 % sucrose gradients containing 20 mM Tris-HCl (pH 7.4 at room temperature), 100 mM KCl, and 30 mM $MgCl_2$. The ribosomal preparations (30 pmol) were centrifuged in a Beckman SW 50 rotor at 48,000 rpm and 4°C for 1.25 h. The distribution of particles along the gradient was determined with an ISCO density gradient fractionator. The proteins obtained from the ribosomal particles by extraction with 67 % (v/v) acetic acid, as well as the split protein fractions, were dialyzed against 0.5 % acetic acid and lyophilized prior to gel electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was performed as described by HOWARD and TRAUT (5). Proteins in the gels were stained with 0.05 % Coomasie Blue, 7.5 % (v/v) acetic acid and 10 % (v/v) ethanol. Protein spots are named according to the nomenclature of KRUISWIJK and PLANTA (2, 6).

Results and Discussion

Modification with DMMA or treatment with NH_4Cl of the 60S subunits from yeast ribosomes is accompanied by dissociation of ribosomal proteins and production of protein-deficient particles which can be separated from the dissociated fraction by centrifugation. The two procedures cause a selective dissociation of certain proteins, while other proteins remain preferentially associated to the residual particles. Figure 1 shows the electrophoretic patterns corresponding to two treatments, one with DMMA

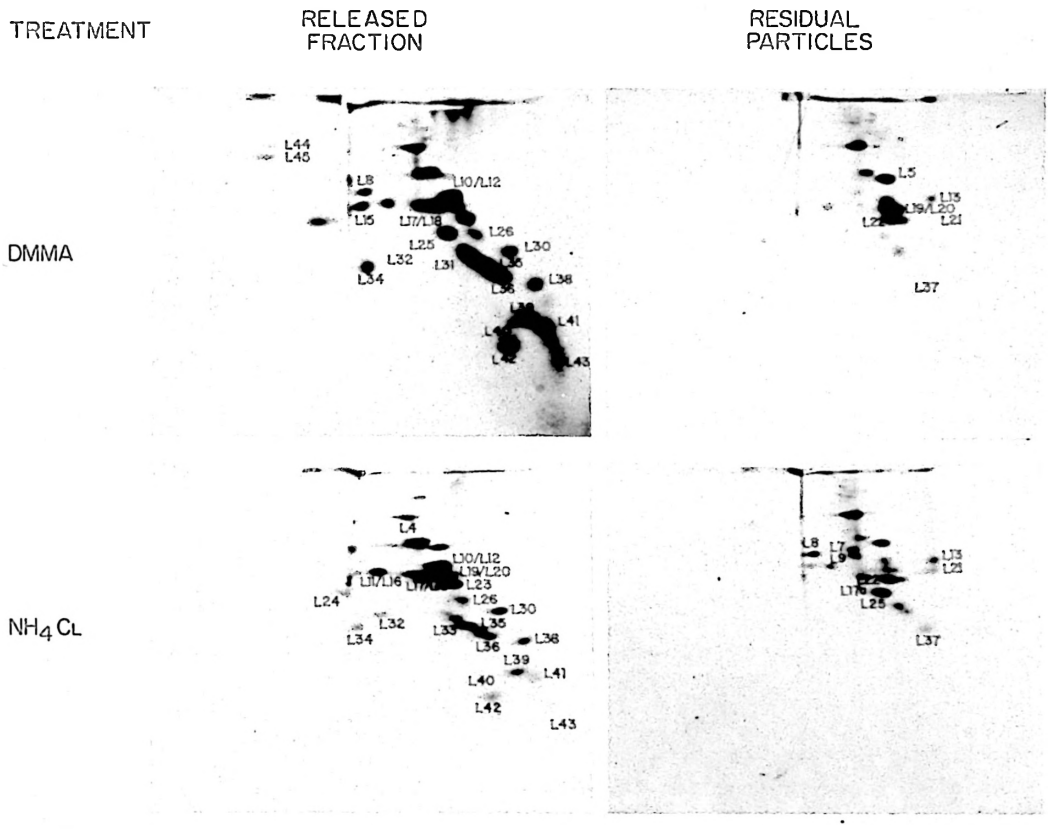

Fig. 1. *Electrophoretic patterns of the proteins released and of those present in the ribosomal particles after treatment with DMMA or NH_4Cl.*
Ribosomal 60S subunits were treated with a molar ratio of DMMA to subunit equal to 24,000 or with 1.5 M NH_4Cl.

and the other with NH_4Cl, which produce the same degree of overall protein dissociation (60 %). Both treatments are accompanied by preferential dissociation of proteins L10/L12, L17/L18, L24, L26, L30, L32, L34, L35, L36, L38, L39, L40/L42, L41 and L43 *. Moreover, NH_4Cl causes the additional selective dissociation of proteins L4, L11/L16, L19/L20, L23 and L33, and DMMA that of L8, L15, L25, L31 and L44/L45. In both types of residual particles, proteins L13, L21, L22 and L37 are present, while L7, L8, L9, L17a and L25 appear only in the NH_4Cl-particles, and L5 and L19/L20 in the DMMA-particles. The most conspicuous differences between the two dissociating procedures are the release by DMMA of L8 and L25, proteins which are present in the residual particles obtained with NH_4Cl, and the liberation of L19/L20 by NH_4Cl and not by DMMA. For treatments that cause low protein dissociation (ratio of DMMA to subunit equal to 2,400, and 1 M

* In our preceding paper (14), spot L5 was erroneously identified as L5/L10/L12, L10/L12 as L19, and L19/L20 as L20.

NH_4Cl), both procedures release proteins L17/L18. In addition, DMMA modification liberates L15, L24 and L44/L45, and NH_4Cl L26. No RNA species was detected in the released fraction obtained with the two procedures, even for the highest treatments used (results not shown), indicating that the 5S and 5.8S RNAs remain bound to the residual particles.

Except for a few proteins, the selectivity of the dissociation produced by the two procedures is similar. Although, in both cases, the electrostatic interactions are directly affected, treatment with NH_4Cl causes a general weakening of ionic forces, while DMMA disturbs the interactions of lysine residues, and at the same time introduces a large number of negative charges. The electrostatic repulsion due to the introduced charges appear to be the main cause of dissociation, since more extensive modification with acetic anhydride produces little release of protein (unpublished results).

The composition of the core particles obtained with DMMA can be varied by modifying the ribosomal particles in the presence of ligands, which could sterically interfere with modification of certain proteins, thus preventing their dissociation, or could affect protein dissociation by inducing changes in ribosome struc-

Table I. *Effects of different ligands on the release by DMMA of certain ribosomal proteins from 60S subunits.*

Ribosomal 60S subunits were incubated, in the modification buffer solution at 37°C for 10 min, in the presence of 0.2 mg elongation factor-2/ml plus 25 μM guanyl-5'-yl methylene diphosphonate (+ EF-2), 4.2 μM ricin A (+ ricin A), 25 μM verrucarine A (+ verrucarine A), 1 mM puromycine (+ puromycine), and in the absence of these compounds. After incubation, the preparations were treated with DMMA at a molar ratio of reagent to subunit equal to 4,800, and the residual ribosomal particles were separated from the released proteins. The proteins of these residual particles were labelled with (^{14}C)-formaldehyde (11), and subjected to two-dimensional gel electrophoresis. The radioactivity of the different protein spots was determined as described previously (9). The values thus obtained are compared with those from a DMMA-untreated control, by assuming that protein L13 is always 100 % present in the residual particles.

Protein Spot	Protein present in the residual particles after DMMA treatment (Percentage of the corresponding protein(s) in the untreated control)				
	Treatment in the absence of ligand	+ EF-2	+ Ricin A	+ Verrucarine A	+ Puromycine
L2/L3	60	59	106	66	90
L4	46	44	102	44	54
L5	54	34	49	58	51
L8	66	16	60	—	33
L11/L16	62	25	77	58	63
L17/L18	33	34	69	43	59
L22	57	29	56	61	53
L23	50	27	35	52	53
L26	42	63	36	20	65
L29	41	36	60	64	47
L32	34	21	56	23	36
L37	42	44	52	54	67
L41	10	21	36	8	29
L43	19	9	42	25	42

ture (9). The effects of elongation factor-2, ricin A, verrucarine A and puromycine on the release of certain ribosomal proteins by modification of 60S ribosomal subunits with DMMA are shown in Table I. The proteins included in the table are those basic proteins the percentage release of which is affected by one of the ligands in at least 20 % of the amount present in native subunits. The presence of elongation factor-2 plus guanyl-5′-ylmethylene diphosphonate decreases the liberation of protein L26, and increases that of L5, L8, L11/L16, L22 and L23. The toxic glycoprotein ricin A (13) protects from the release of proteins L2/L3, L4, L17/L18, L32, L41 and L43. Verrucarine A, a trichothecene antibiotic (13), protects L29 from dissociation, while increasing the release of L26. Puromycine, an inhibitor of peptide-bond formation (13), facilitates the liberation of L8, and protects L2/L3, L17/L18, L26, L37 and L43 from dissociation. Since the protective effect of a ligand against the release of specific proteins is probably caused by its presence at the binding site, where it interferes sterically with modification (9), the ribosomal proteins protected from release might be located at the ligand binding site or close to it.

The protein-deficient particles obtained from eukaryotic ribosomes by treatment with monovalent cations were found to have decreased sedimentation rates as compared to the original particles (3, 10), which is consistent with protein loss and structural relaxation. The sedimentation of the core particles prepared with DMMA can be determined before and after regeneration of the modified amino groups, which allows a gross evaluation of the structural change due to the presence of the negatively-charged reagent moieties. The sedimentation patterns of the residual particles obtained with different amounts of DMMA, before and after regeneration of the modified amino groups, are shown in figure 2. For a molar ratio of reagent to subunit of 1,200, no significant change in sedimentation is observed, while for higher amounts of DMMA a decrease in sedimentation rate is found. In all cases, the residual particles appear fairly homogeneous. The particles prepared with a molar ratio of DMMA to subunit of 4,800 have a sedimentation coefficient, S_{20}, of 32S, as determined in the analytical ultracentrifuge. For all treatments used, no detectable change in the sedi-

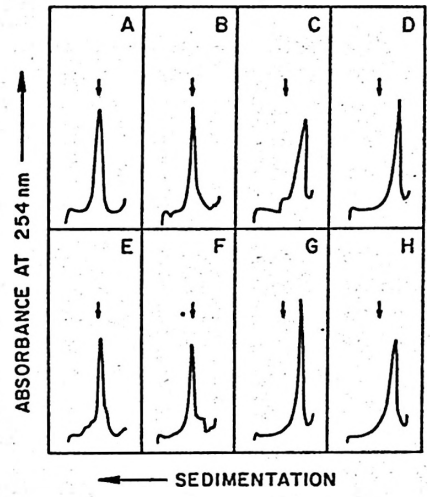

Fig. 2. *Sedimentation patterns of protein-deficient ribosomal particles obtained by treatment with different amounts of DMMA.* Ribosomal 60S subunits were treated with DMMA at the following molar ratios of reagent to subunit: 0 (A, E), 1,200 (B, F), 2,400 (C, G), and 4,800 (D, H). The percentage release of protein was: 0 (A, E), 15 (B, F), 20 (C, G), and 40 (D, H). The released proteins were separated by centrifugation, and the sedimentation patterns of the residual particles were obtained before (A, B, C, D) and after (E, F, G, H) incubation with 3 volumes of 50 mM cacodylate (pH 6.0), 80 mM KCl and 12.5 mM $MgCl_2$, treatment that regenerates the modified amino groups. The arrows in the sedimentation patterns indicate the position of the original 60S subunits.

mentation rate of the residual particles is found after regeneration of the modified amino groups. These results indicate that the reagent moieties produce no gross effect on the sedimentation rate of the residual particles.

In spite of the apparent homogeneity observed in sedimentation, the protein-deficient particles obtained by the two procedures, although devoid of some proteins, show a significant degree of heterogeneity, since a substantial number of individual proteins are distributed between the released fraction and the residual particles.

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Resumen

Se comparan dos procedimientos distintos de obtención de partículas ribosómicas deficientes en proteínas a partir de subunidades 60S de levadura: la modificación reversible de los residuos de lisina con anhídrido dimetilmaleico, y el tratamiento con NH_4Cl. Los dos procedimientos causan la disociación selectiva de ciertas proteínas. Con algunas excepciones, la especificidad de la disociación es semejante en ambos casos. Cuando se usa el anhídrido dimetilmaleico, se producen cambios en la composición de las partículas residuales al modificar las subunidades ribosómicas en presencia de alguno de los siguientes compuestos: factor de elongación 2, ricina A, verrucarina A y puromicina.

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