ANALYSIS OF THE PROTEIN COMPOSITION OF YEAST RIBOSOMAL SUBUNITS BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

T. KRUISWIJK AND R. J. PLANTA

Biochemisch Laboratorium, Vrije Universiteit, De Boelelaan 1085,
Amsterdam-Buitenveldert, The Netherlands

(Received 25 July, 1974)

ABSTRACT. The number of proteins in yeast ribosomal subunits was determined by two-dimensional polyacrylamide gel electrophoresis. The 40S subunit obtained after dissociation of ribosomes at high ionic strength contains 30 different protein species (including six acidic proteins). The 60S subunit, obtained in the same way contains 39 different species (including 1 acidic protein). While the total number of protein species found in yeast ribosomes, thus, is in close agreement with those reported for other eukaryotic organisms, the distribution between acidic and basic proteins is quite different.

When the ribosomes were dissociated at low ionic strength, four extra protein spots appeared in the electropherograms of both 40S and 60S subunits. We consider these proteins to be non-ribosomal.

I. INTRODUCTION

Characterization of ribosomal proteins is a prerequisite for the analysis of structure and function of the ribosome. Up till now most efforts towards analysing ribosomal structure and function have concentrated on prokaryotic organisms [1] while relatively little is known about eukaryotic ribosomes. The eukaryotic ribosome contains approximately 70 different polypeptide chains [2-13], whereas for instance in E. coli ribosomes only 55 different proteins are present [1]. A start has been made on the characterization of the proteins from eukaryotic ribosomes [2-13]. The results so far obtained indicate that the ribosomal proteins from eukaryotic cells have the same general characteristics as those from prokaryotic organisms. Eukaryotic ribosomes, however, appear to contain a larger number of individual proteins than do bacterial ribosomes.

It is a matter of speculation whether the larger number of proteins present in eukaryotic ribosomes has anything to do with the process of cell differentiation, occurring in higher organisms. Comparison of ribosomal proteins from various species or from various organs within a given species [14-17], suggests that for a given organ, ribosomal proteins do not vary according to the stage of differentiation [15]. However within a given species the protein composition of the ribosomes is, to a certain extent, tissue-specific [17]. The differences in ribosomal proteins between various species can be quite extensive [14, 16]. It was, therefore, of interest to examine the ribosomal protein composition of a primitive, eukaryotic organism like yeast.

Furthermore, knowledge of the number of single proteins present in eukaryotic ribosomes and
their properties is vital for a study of ribosome assembly in eukaryotes. Since the synthesis of ribosomes in yeast has been studied extensively in our laboratory [18], the analysis of the ribosomal proteins in this organism is a further step in unraveling the complicated process of ribosome assembly and ribosome function.

Therefore we initiated a two-dimensional electrophoretic analysis of ribosomal proteins from the yeast Saccharomyces carlsbergensis, the first results of which are described in this communication.

II. EXPERIMENTAL

Saccharomyces carlsbergensis, strain S74, was grown at 30°C in a normal medium containing per liter: 10 g of glucose, 5 g of bacteriological peptone, 3 g of yeast-extract and 3 g of malt-extract. Growth was followed by measuring the turbidity of the culture at 550 nm. The cells were harvested by centrifugation when the A_550 reached a value of 0.5, washed once with aqua bidest and suspended in buffer A (10 mM Tris-HCl pH 7.4, at 25°C, containing 10 mM KCl, 10 mM MgCl₂, 5 mM β-mercaptoethanol, 0.5% Brij-58 and 3 ml macaloid suspension per liter). The suspension was shaken with glass beads in a Braun Shaker during 45 sec at 4°C.

Total ribosomes were isolated from the homogenate by means of centrifugation: during 30 min at 30000 x g in the 60-Ti-rotor (Spinco, Beckman) followed by centrifugation of the supernatant for 120 min at 150000 x g in the 60-Ti-rotor.

The ribosomal pellet was suspended in dissociation buffer B (10 mM Tris-HCl pH 7.4, at 25°C, containing 60 mM KCl, 0.01 mM MgCl₂, 5 mM β-mercaptoethanol and 3 ml macaloid suspension per liter) or dissociation buffer C (10 mM Tris-HCl pH 7.4, at 25°C, containing 0.5 M KCl, 10 mM MgCl₂, 5 mM β-mercaptoethanol and 3 ml macaloid suspension per liter).

Ribosomes suspended in buffer B were layered directly on sucrose gradients while those suspended in buffer C were incubated with 0.2 mg puromycin for 30 min at 0°C and then for another 15 min at 37°C [19] prior to separation of the subunits. 200 mg ribosomes in 10 ml buffer B or C, containing 5% (w/v) sucrose, were layered on a linear sucrose gradient, 10–40% (w/v), in buffer B or C, followed by 100 ml overlayer (buffer B or C) and the ribosomal subunits were separated by centrifugation in a B-XIV zonal rotor (Spinco, Beckman) at 48000 rpm for 200 min at 8°C. The gradient was displaced with 50% (w/v) sucrose in aqua bidest and collected in 12 ml fractions. The fractions were pooled according to the type of subunits they contained, and the particles were collected by centrifugation for 18 hr at 4°C in a 60-Ti-rotor (Spinco, Beckman) at 60000 rpm.

The colourless pellets were suspended in buffer D (10 mM Tris-HCl pH 7.4, at 25°C, containing 0.1 mM MgCl₂, 5 mM B-mercaptoethanol and 0.1 mM phenylmethylsulfonylfluoride (PMSF). Protein extraction was performed by adding 5 volumes of ice-cold 2-chloroethanol, followed by HCl to a final concentration of 60 mM [20]. After incubation for 3 hr at 4°C, the RNA was collected by low speed centrifugation. The RNA pellet was extracted once more overnight. The combined supernatant fractions were pooled and dialyzed against distilled water for 1 hr, and subsequently against a solution containing 1 mM HCl, 3 mM β-mercaptoethanol and 0.1 mM PMSF for at least 24 hr. The protein solutions were lyophilized and the residues stored at -30°C.

The ribosomal proteins (1.0–1.6 mg in 50–100 μl of a solution containing 9 M ureum, 1 mM dithiotreitol, 25% (w/v) sucrose) were analysed by two-dimensional gel electrophoresis according to Kaltaschmidt and Wittmann [21]. The standard conditions of electrophoresis were: 4% polyacrylamide, pH 8.6, for 24 hr at 120 Volts in the first dimension; 18% polyacrylamide, pH 4.6, for 24 hr at 140 Volts in the second dimension. All runs were performed at 4°C. The gels were stained in 0.1% Coomassie Brilliant Blue for 2.5 hr and destained in a solution containing 5% acetic acid and 8% methanol.