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# CHARACTERIZATION OF WHEAT ROOT RIBOSOMES ISOLATED BY $Mg^{2+}$ PRECIPITATION

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**Key Word Index**—*Triticum vulgare*; Gramineae; wheat; ribosomes, $Mg^{2+}$ precipitation; ribosomal proteins; phosphorylation *in vitro*.

**Abstract**—The $Mg^{2+}$ precipitation method has been adapted for isolation of ribosomes from roots of wheat. The ribosomes prepared by this procedure show $A_{260}/A_{280} = 1.6$ and $A_{260}/A_{235} = 1.3$ and contain 44% RNA and 56% ribosomal proteins. There are no detectable differences in the ribosomal protein complement and accessibility of the ribosomal proteins to phosphorylation between ribosomes isolated by this procedure and those prepared by classical ultracentrifugation methods. The ribosomes are active in a poly-U directed cell-free system for protein synthesis.

## INTRODUCTION

The importance of $Mg^{2+}$ for the structural and functional integrity of the ribosomes is well known [1]. $Mg^{2+}$ has a stabilizing effect on the subunit interactions and (at lower concentrations) on the internal structure of the subunits [2–6]. Increase of the $Mg^{2+}$ concentration above a certain value results in precipitation and aggregation of the ribosomes [7,8]. The mechanism of the precipitation reaction however is unknown. The precipitation of the ribosomes by $Mg^{2+}$ has been used in procedures for the isolation and purification of ribosomes from animal tissues [9–11]. Diespenger *et al.* [12] have described a procedure for the rapid isolation of a microsomal fraction from parsley cells which they characterized by the marker enzyme activity.

We have applied the $Mg^{2+}$ precipitation for the isolation of ribosomes from roots of wheat. We have shown that ribosomes obtained by this procedure according to their chemical characterization are indistinguishable from those isolated by ultracentrifugation. The ribosomes are active in a poly-U directed cell-free system for protein synthesis.

## RESULTS

### *Optimal conditions for $Mg^{2+}$ precipitation of wheat root ribosomes*

Increase of the concentration of $Mg^{2+}$ in wheat root postmitochondrial supernatant to 10 mM causes aggregation and precipitation of about 9.4% of the total ribosomes. Maximum precipitation of ribosomes can be achieved by incubation of the postmitochondrial supernatant for 1 hr at 0° at 50 mM final concentration of $Mg^{2+}$. The recovery expressed in $A_{260}$ units/g (fr. wt) roots is 6.3, which is 94% of the yield obtained by ultracentrifugation (6.7 $A_{260}$/g roots). An increase of $Mg^{2+}$ concentration to 60 mM does not lead to a greater recovery but at 100 mM $Mg^{2+}$ there is a decrease in the $A_{260}$ yield to 75,2%. The ribosomal aggregates sediment completely on centrifugation at 25 000 rpm for 90 min. High RNase activity of the root homogenate has been detected in preliminary experiments. 2-Mercaptoethanol (5 mM) increases the stability of the wheat root ribosomes while bentonite has no effect. Isolation was therefore carried out in the presence of 2-mercaptoethanol.

### *Characterization of the ribosomal preparation*

A typical preparation showed $A_{max}$ at 260 nm and $A_{260}/A_{280} = 1.6$ and $A_{260}/A_{235} = 1.3$ which are somewhat lower than those of pure ribosomes [1]. The RNA/protein ratio is 44%:56% which is similar to that of the most plant ribosomal preparation which usually contain about 40% RNA and 60% protein [1,13,14]. The purification of the ribosomes by $Mg^{2+}$ precipitation as well as by centrifugation through a sucrose cushion containing 0.5 M KCl does not change these values.

### *Properties of the ribosomal RNA*

RNAs extracted from the ribosomal pellet were separated by agarose gel electrophoresis. Two main bands corresponding to 25S and 18S ribosomal RNAs and a third one with mobility of 4–5S RNAs are detected. Co-electrophoresis with RNA extracted from ribosomes isolated from wheat roots by ultracentrifugation does not show any additional electrophoresis band.

### *Ribosomal protein*

The total protein content of the ribosomes isolated by $Mg^{2+}$ precipitation is in the normal range. We cannot detect any significant difference between the 2D polyacrylamide gel electrophoresis patterns of proteins from ribosomes isolated by $Mg^{2+}$ precipitation and those isolated by ultracentrifugation [15]. This result shows that the presence of a high $Mg^{2+}$ concentration during the isolation procedure of the ribosomes does not change the total protein content and the number of ribosomal

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proteins but it may induce the ribosomes to form a more compact structure. If this is so, some changes in the localization of the ribosomal proteins on the surface of the ribosomes and their accessibility to chemical modification should be detected. For this reason we studied the phosphorylation *in vitro* of the ribosomes isolated by $Mg^{2+}$ precipitation. Five ribosomal proteins are strongly phosphorylated under these conditions. Comparison of these data with phosphorylation patterns of the ribosomes isolated by ultracentrifugation does not reveal differences as far as the strongly labelled spots are concerned [15].

*Biological activity of the ribosomal preparation*

The ribosomes isolated by $Mg^{2+}$ precipitation are biologically active as tested in the poly-U directed polyphenylalanine synthesising system. The time course of the incorporation of phenylalanine-[$^{14}$C] is linear up to 1 hr of incubation. The maximal incorporation is 9.24 pmol amino acid/10 $A_{260}$ units ribosomes per min.

## DISCUSSION

The present results show that the $Mg^{2+}$ concentration and the presence of a ribonuclease inhibitor during the procedure of isolation are important for maximum recovery of ribosomes from wheat roots by $Mg^{2+}$ precipitation. The chemical characteristics of the ribosomes isolated by this procedure are within the range of those determined for plant ribosomes isolated by classical ultracentrifugation methods. The presence of a high $Mg^{2+}$ concentration during the preparation does not cause any detectable changes in the protein content, ribosomal protein complement and location of the ribosomal proteins, as judged by their accessibility to chemical modification. The ribosomes are active in a poly-U directed cell-free system for protein synthesis. The $Mg^{2+}$ precipitation appears to be a suitable method for isolation of native ribosomes from wheat roots. The main advantage of this method is that it avoids ultracentrifugation and it could be useful when large amounts of ribosomes are needed to be isolated.

## EXPERIMENTAL

*Isolation of ribosomes by $Mg^{2+}$ precipitation.* Root tips (70–80 g) from 72-hr-old etiolated wheat seedlings were ground in pre-chilled mortar with 3 vol of 250 mM sucrose containing 5 mM $MgCl_2$, 50 mM KCl, 5 mM 2-mercaptoethanol, 25 mM Tris–HCl, pH 7.5. The homogenate was filtered through cotton cloth and centrifuged for 30 min at 15000 rpm. The post-mitochondrial supernatant was adjusted to 2% with respect to Triton X-100 and M $MgCl_2$ was added to a 50 mM final conc. The mixture was incubated for 1 hr at 0° and the ribosomes sedimented by centrifugation at 25000 rpm for 90 min. Ribosomes were purified either by further reprecipitation or by passing through a sucrose cushion consisting of M sucrose, 500 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 10 mM Tris–HCl, pH 7.5.

*Characterization of ribosomes.* Protein content was determined by the procedure of ref. [17] and RNA content by the orcinol method [18]. RNAs were extracted by the pHOH/SDS procedure and fractionated by agarose gel electrophoresis [19]. The dried electrophoretic plates were traced. Ribosomal proteins were extracted with 66% HOAc, lyophilyzed and fractionated by 2-D polyacrylamide gel electrophoresis as described in ref [15]. Phosphorylation of the ribosomes was performed by ATP [$\gamma$-$^{32}$P] in a cell-free system [15]. Cell sap passed through a Sephadex G-25 column was used as source of a protein kinase. The phosphoproteins were detected by autoradiography of the 2-D electrophoretic plates.

*Determination of the biological activity of the ribosomes.* The poly-U directed cell-free system for protein synthesis contained in a total vol of 0.3 ml: 50 mM Tris–HCl, 75 mM KCl, 15 mM $MgCl_2$, 2 mM ATP, 0.2 mM GTP, 1 mM 2-mercaptoethanol, 50 $\mu$g poly U, 10 mM PEP, 40 $\mu$g/ml pyruvate kinase, 1 $\mu$Ci phenylalanine-[$^{14}$C] (271 Ci/mol), 10 $A_{260}$ units ribosomes, cell sap corresponding to 0.25 mg protein at pH 7.5 [20,21]. After incubation for different periods at 37° aliquots from each tube were absorbed on Whatman 3 MM filter discs. Radioactivity was determined by the method of refs [22] and [23].

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