Purification of eukaryotic translation factors from wheat germ for reconstitution of protein synthesis

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ABSTRACT

The wheat germ cell-free protein synthesis is a powerful and versatile method for preparation of proteins based on the accumulated DNA sequence information. As the cell extract used for it contains many factors that are unknown or do not directly involve in protein synthesis, details of the translation reaction is yet to be understood. Therefore, we have decided to try reconstitution of protein synthesis, which would be useful for better understanding of the mechanisms supporting eukaryotic protein synthesis and translational regulation and probably applicable to synthetic biology. In the present study, we fractionated an extract from crude wheat germ according to published protocols to obtain the fractions containing the eukaryotic elongation factors (eEFs) 1A, 1B, and 2. The eEF1A and eEF2 fractions supported polyphenylalanine synthesis.

INTRODUCTION

After the genome sequencing projects, elucidation of the interactions of the gene products that constitute complex networks supporting all activities of the cells is a major issue for understanding life. In particular, eukaryotic cells have developed very complex regulation systems that may cause differentiation, cancer, cell death, and so on. The wheat cell-free protein synthesis system (4) is a very powerful tool for preparation of proteins used for experimental approaches to the understanding of protein networks (5). On the other hand, cytosolic protein synthesis is involved in many of the cellular regulation events especially in eukaryotes. As the protein synthesizing S30 extract is derived from extensively washed wheat embryos that are free from endosperm materials, the ribosomes and the translation apparatus are quite robust during long incubation periods(4). Thus, wheat may be the most promising source that could be used for the investigation of the regulation mechanisms mediated by the translation machinery by constitutive or synthetic biology approaches.

However, the cell extract used for the wheat cell-free translation contains unknown molecules and those that are not involved in protein synthesis. It also contains mRNA degradation enzymes that complicate the protein synthesis reaction. In addition, understanding of the mechanisms in eukaryotic protein synthesis reactions, including initiation, elongation, termination, polysome formation and disassembly, protein folding and subunit joining, and so on,

![Graph showing KCl concentration and absorbance over time](image-url)

Figure 1. DE52 chromatography of the 40-70% saturated ammonium sulphate fraction from the S170 supernatant of the wheat germ extract. Fractions were eluted at the KCl concentrations shown above, and the absorbance was monitored at 280 nm. The fractions containing the elongation factors are shown by arrows.
is still incomplete.

Therefore, we decided to try reconstitution of protein synthesis based on the apparatus from wheat. As the first step, in the present study, we followed the protocols established more than 20 years ago for purifying elongation factors (1).

RESULTS AND DISCUSSION

Crude wheat germ from a flouring mill was fractionated essentially according to the literature (1) as follows. An S30 extract was prepared from the wheat germ and was centrifuged for 3.5 h at 170,000 g. The 40-70% saturated ammonium sulphate fraction of this supernatant was fractionated by DE-52 column chromatography (Fig. 1). The eEF1A and eEF2 fractions were further separated by ammonium sulphate precipitation and P11 column chromatography. The eEF1A fraction protected an aminoacyl-tRNA from deacylation (Fig. 2). The eEF2 fraction supported polyphenylalanine synthesis with the ribosomes and the eEF1A fraction (Fig. 3).

At this stage, the purities of the eEF fractions are not sufficient for reconstitution experiments, as judged from gel electrophoresis patterns. However, we are trying some other methods for further purification of the factors. We have already found that a fraction that seems to contain eEF1A and eEF1B almost exclusively could be obtained by a single step chromatographic separation after ammonium sulphate fractionation. Eukaryotes use many different initiation factors for specific initiation of translation. Thus, we are testing mRNAs with some internal ribosome entry sites and leaderless mRNAs, which are reported to bypass the factor-dependent initiation process (2,3). Fractionation and reconstitution of wheat translation components was tried in 1980's, but, at that time, many translation factors were unknown. Most of the factors and the source of the possible instability of the ribosomes are now known. Techniques for chromatographic separation of proteins, in general, have also been improved. Therefore, we expect that it is easier now to reconstitute protein synthesis.

REFERENCES


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