CELL-FREE BIOSYNTHESIS OF LIPOAMIDE DEHYDROGENASE

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Introduction

Lipoamide dehydrogenase (NADH-lipoamide oxidoreductase, EC 1. 6. 4. 3) is an essential component of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase multienzyme complexes (1). Previously, we demonstrated that the two forms of the lipoamide dehydrogenase separated from the pig heart mitochondrial multienzyme complexes are functionally interchangeable, and identical in molecular weight, dimeric structure, amino acid composition and immunochemical properties (2). These results suggest that the mammalian lipoamide dehydrogenase is also the product of a single structural gene just like Escherichia coli.(3). We are interested in the regulation of biosynthesis of this common component enzyme of two species of the multienzyme complexes. In this report, we describe the purification of the lipoamide dehydrogenase mRNA by polysome immunoadsorption method, and the cell-free biosynthesis of the lipoamide dehydrogenase in the reticulocyte lysate system.

Experimental Procedures

Enrichment of Lipoamide Dehydrogenase mRNA---Total polysomes isolated from the pig liver by the Mg-precipitation method (4), were incubated with monospecific anti-lipoamide dehydrogenase IgG (2.5mg/1,000A_{260}) purified by antigen-Sepharose

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chromatography (5), at 0°C for 3h. The antibodies bound to polysomes were adsorbed to the protein A-Sepharose column (6). After washing, enriched polysomal RNA was eluted, deproteinized by protease K treatment and poly(A) RNA was isolated by oligo(dT)-cellulose chromatography.

Cell-Free Translation and Immunoprecipitation---Staphylococcal nuclease-treated rabbit reticulocyte lysate was prepared as described by Pelham and Jackson (7), and *in vitro* translation was carried out by the method of Mori *et al*. (8). Incubation was carried out at 25°C for 90 min. Immunoprecipitation of the translation product was performed by using anti-lipoamide dehydrogenase IgG and protein A-coated *Staphylococcus aureus* cells (9). SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (10) by using 10% gel and fluorographed by using Amplifier (Amersham).

Results and Discussion

Lipoamide dehydrogenase mRNA was found in the pig liver at a very low level (i.e. less than 0.01% of the total cellular mRNA as measured by an *in vitro* translation assay). Therefore, it was necessary to concentrate by several enrichment procedures to obtain sufficient amounts of mRNA for translation or cDNA cloning. The lipoamide dehydrogenase mRNA was purified by immunoadsorption of polysomes isolated from the one month old pig liver. Translation product with the purified mRNA was precipitated by antibody. Analysis of the immunoprecipitate by SDS-gel electrophoresis revealed a 53K band and an additional 55K band which was co-migrated with the mature lipoamide dehydrogenase as shown in Fig. 1, lanes 1 and 3. These bands were not observed in the translation products with the crude liver RNA, however, they were observed in the products with the purified mRNA (lane 2). Furthermore these bands were not detected in the precipitates by using the control IgG from non-immune rabbit.

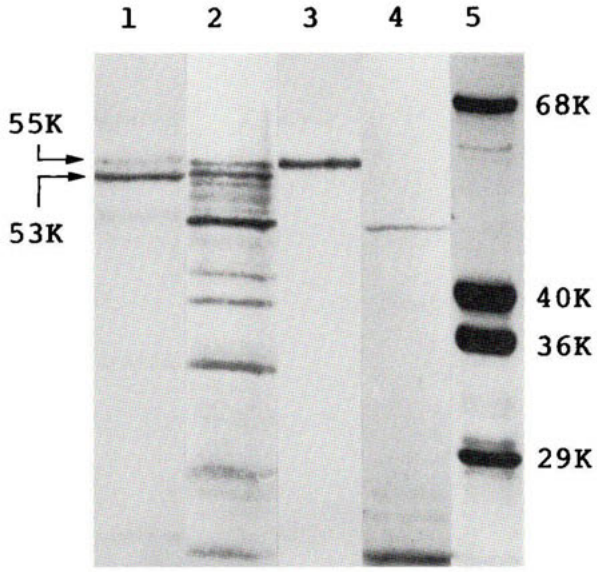

Fig. 1. Electropherograms of the lipoamide dehydrogenase subunits synthesized in vitro by the purified mRNA. The purified mRNA (0.5 µg) was translated at 25°C for 90 min in a final volume of 10 µl of the reticulocyte lysate system containing 2 µCi of [^{35}S]methionine. Translation products were analyzed by the immunoprecipitation and SDS-gel electrophoresis. Lane 1 shows the immunoprecipitated products; lane 2, total products; lane 3, mature ^{14}C-methylated lipoamide dehydrogenase subunit; lane 4, products without exogenous mRNA; and lane 5, ^{14}C-methylated marker proteins (bovine serum albumin, aldolase, glyceraldehyde 3-phosphate dehydrogenase, and carbonic anhydrase). Arrows indicate the lipoamide dehydrogense subunits (55K & 53K) synthesized.

Recently, it is thought that most of the mitochondrial protein coded by nuclear gene, are synthesized as a larger precursor on cytoplasmic polysomes. However, the size of the lipoamide dehydrogenase synthesized in vitro was not larger than the mature form. This could be due to a number of factors such as premature termination of translation, cleavage of the mRNA either during isolation or translation by nuclease, partial degradation of the primary translation product and an abnormal behavior of the product on SDS-gel electrophoresis.

The comparison of radioactivities of the immunoprecipitated translation products by using both crude RNA and the purified mRNA indicated about 50 to 100-fold enrichment. We believe that this enriched lipoamide dehydrogenase mRNA would be sufficient to prepare corresponding cDNA for cloning or screening purposes.

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