STUDIES ON PROTEINS OF ANIMAL RIBOSOMES. XIV. ANALYSIS OF PHOSPHORYLATED RAT LIVER RIBOSOMAL PROTEINS BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

J.STAHL, H.WELFLE and H.BIELKA

Central Institute of Molecular Biology of the German Academy of Sciences of Berlin, Department of Cell Physiology, 1115 Berlin-Buch, Germany

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1. Introduction

Phosphorylation of proteins in ribosomes and ribosomal subunits and of isolated proteins seems to be a possibility to get some information on the topography of proteins in ribosomal particles.

Kabat [1,2] and Loeb and Blat [3] have shown that some proteins of animal ribosomes can be phosphorylated *in vivo* and *in vitro*. Subunits of rat liver ribosomes have been phosphorylated *in vitro* by incubation with protein kinase and [γ-^{32}P] ATP by Eil and Wool [4]. They found after one-dimensional polyacrylamide gel electrophoresis of the isolated proteins three proteins labelled in the small subunit preparation and nine in the large one.

In the following we describe the results of *in vitro* phosphorylation of proteins of rat liver ribosomes, their subunits and their isolated proteins obtained by two-dimensional polyacrylamide gel electrophoresis and autoradiography. Proteins organized in ribosomal particles are less accessable to phosphorylation than isolated ribosomal proteins.

2. Materials and methods

Ribosomes, ribosomal subunits and ribosomal proteins were prepared as described in a preceding paper [5].

Phosphorylation was carried out in a buffer containing 20 mM Tris-HCl, pH 7.2, 0.002 mM cyclic AMP, 5 mM $MgCl_2$, 2 mM theophylline, 0.1 mM

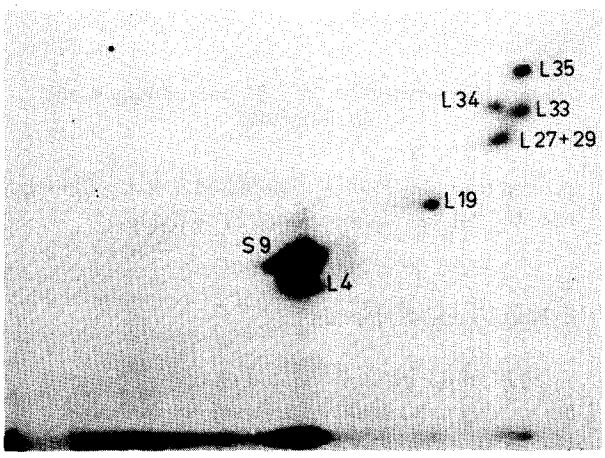

Fig. 1. Radioautographs of proteins from phosphorylated whole ribosomes.

ethyleneglycol bis-(aminoethyl)-tetraacetic acid. About 2 mg of ribosomal particles or ribosomal protein per ml were incubated for 30 min at 30° with 200 μg protein kinase purified from beef skeletal muscle by DEAE-cellulose chromatography according to Mijamoto et al. [6] and with [γ-^{32}P]-ATP in concentrations of about 0.02 mM. [γ-^{32}P] ATP was prepared by a simplified method of Gibbs et al. [7], yielding specific activities of 1–5 Ci/mmole. After incubation the phosphorylation reaction was stopped by adding 0.25 N HCl in the cold and the proteins were prepared as described earlier [5].

The proteins were separated by two-dimensional electrophoresis [5] and the stained gels slabs were

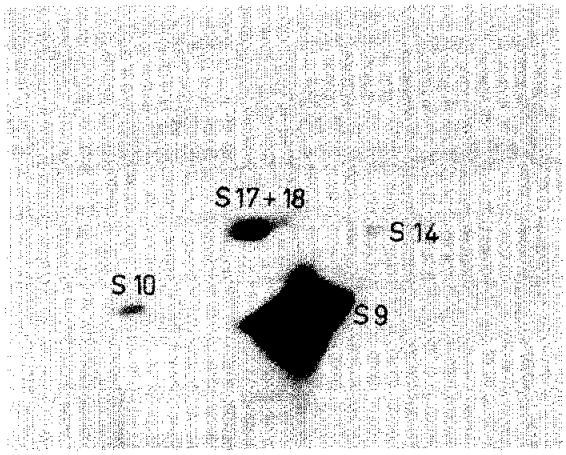

Fig. 2. Radioautographs of proteins from phosphorylated small subunits.

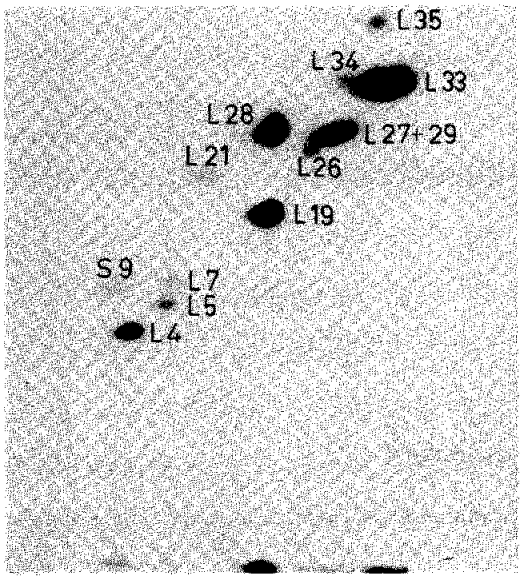

Fig. 3. Radioautographs of proteins from phosphorylated large subunits.

packed into plastic foils, covered with X-ray films and exposed for various periods (6 hr to 6 days).

3. Results and discussion

Most of the phosphorylated ribosomal proteins are moving cationically. Furthermore, we found two weak anionically moving labelled components of unknown origin, both being not in coincidence with the Amidoblack-stained proteins L40 and L41.

Phosphorylation reduces the cationic mobility of labelled ribosomal proteins to varying extent during electrophoresis in the first dimension. Because the mobility in the second dimension, in which the proteins are separated mainly due to their molecular weights, does not seem to be altered significantly, the labelled spots can be fairly well correlated to the Amidoblack pattern. In the case of closely neighboured spots it is not always possible to correlate them precisely, because of the limited resolution efficiency of the autoradiographs. Furthermore, the spots of very low intensity have not been taken into account.

Figs. 1–3 show the labelled spots in two-dimensional patterns of proteins prepared from phosphorylated particles. When whole ribosomes are phosphorylated mainly the protein S9 is labelled. Besides, the proteins L4, L19, L27 + 29, L33, L34 and L35 are labelled to a considerably smaller extent (fig.1).

By phosphorylation of the small subunit the protein S9 is labelled with extremely high intensity (fig.2). Compared with the pattern of total ribosomes in addition S10, S14 and S17 + 18 are labelled.

When large subunits are subjected to phosphorylation in addition to the proteins already accessable to phosphorylation in the whole ribosome (L4, L19, L27 + 29, L33, L34, L35), especially the protein L29 becomes remarkably labelled besides the components L5, L7, L21 and L26 which are of lower radioactive intensity (fig.3). Furthermore it is evident from this pattern that even very weak contaminations of the large subunit preparation with the small subunit become visible because of the high accessability of the protein S9 to phosphorylation.

Figs. 4–6 show the spots being labelled when isolated proteins are phosphorylated and analysed by two-dimensional electrophoresis. The number of labelled components is higher than in experiments with the corresponding particles.

When the proteins isolated from the small subunit

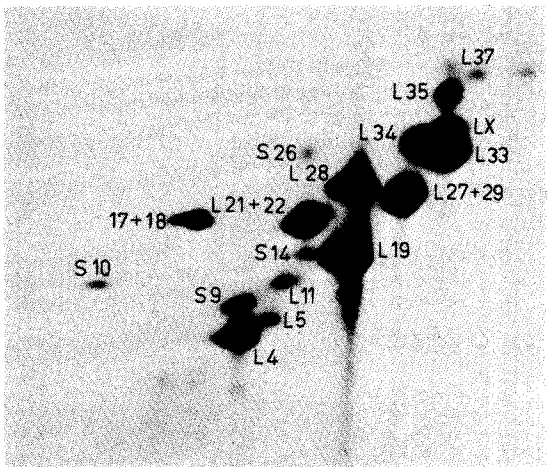

Fig. 4. Radioautographs of isolated total protein.

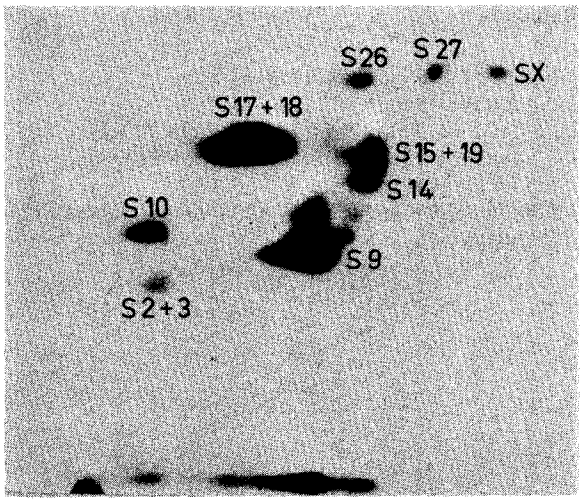

Fig. 5. Radioautographs of isolated small subunit protein.

are phosphorylated (fig.5), in addition to S9, S10, S14 and S17 + 18, which are also phosphorylated in the small subunit, the spots S2 + 3, S15 + 19, S26 and S27 become labelled. A further spot, designated as Sx, cannot be correlated to any of the Amidoblack spots.

The tendency of increasing accessability to phosphorylation when isolated proteins instead of particles are phosphorylated is also obvious in the case of the large subunit (fig.6). In addition to the spots already labelled in the particles, the spots L21 + 22 and L37 become visible. Similar to the phosphorylation pattern of the small subunit protein, also a spot of unknown origin, designated as Lx, appears after phosphorylation of the isolated protein of the large subunit.

The phosphorylation pattern of total ribosomal protein is given in fig.4. About 16 proteins are visible in the pattern, which is, for most of the spots, in accordance with the patterns of the labelled subunit proteins. The identification of some spots, in particular of S2 + 3, S15 + 19 and S27 is difficult, because the intensity of the small subunit proteins is relatively low and the positions of some spots are covered by neighboured intensive spots of the large subunit.

It is evident that even isolated ribosomal proteins, which most probably all contain serine- and threonine-residues [8], are phosphorylated only in part. As already mentioned before, when proteins are organized in small and large subunits, the number of proteins accessable to phosphorylation, is reduced. Association of small and large subunits to whole ribosomes covers further proteins, which cannot be phosphorylated anymore.

Acknowledgements

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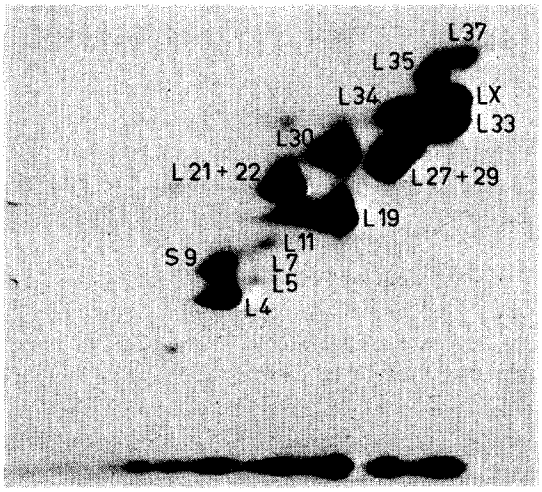

Fig. 6. Radioautographs of isolated large subunit protein.

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