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The Kinetics of ^{32}P Incorporation into Brain and Liver Ribosomal Proteins from 14-Day Old Chick Embryos

Kinetyka inkorporacji ^{32}P do białek rybosomalnych mózgu i wątroby 14-dniowych zarodków kurzych

Кинетика инкорпорации ^{32}P в рибосомальные белки мозга и печени 14-дневных куриных эмбрионов

The phosphorylation of eucariotic ribosomal proteins, *in vivo* has been demonstrated in a number of different tissues (9, 3, 2, 19, 14). The rate of ribosomal protein phosphorylation decreased in thyroidectomized rats (5), but increased in glucagon-injected rats (4) and in the actively dividing cells (6, 15, 10). Despite these findings, the function of phosphorylation in the cellular metabolism remains unknown.

The brain and the liver of 14-day old chick embryos differ in mitotic activity, thus it was interesting to compare the kinetics of ^{32}P incorporation into ribosomal proteins of both the tissues. Some results have been presented in a preliminary report (17).

MATERIALS AND METHODS

For each experiment, fifty 14-day old chick embryos were used. Radioactive phosphate 80 μCi of ^{32}P Na_2HPO_4 (spec. act. 330 mCi/mmol), obtained from the Institute of Nuclear Research (Świerk, Poland), in 0.1 ml of 0.9% NaCl solution, was introduced intraamnionally. After 4, 10 or 20 h the embryos were decapitated and the brains and livers were isolated.

From the brains and livers the ribosomes were prepared, extracted with 0.25 N HCl and proteins were precipitated with trichloroacetic acid, as described by Sanecka-Obacz and Borkowski (16), with the only difference that the ribosomes were washed twice with buffered 0.5 N KCl and 1% Triton X-100.

The proteins were fractionated on a column of CM-cellulose (CM-52 Whatman, pre-swollen). Protein, 2—3 mg, in acetate buffer *pH* 4.2 was applied to the column (0.9×6 cm) and eluated successively with 0.01 and 0.02 N HCl. Fractions of 3 ml were collected at a flow rate of 0.5 ml/min and extinction at 278 nm was determined.

Polyacrylamide-gel electrophoresis was performed according to Johns (8), using 20% gel. Densitometry was performed with a Kipp and Zonnen densitometer.

Protein was determined according to Lowry et al. (11) with bovine serum albumin as a standard, and phosphorus — by the method of Hurst (7).

Phosphoserine and phosphothreonine in the proteins were detected electrophoretically following mild acid hydrolysis (18).

Radioactivity was measured in a sample containing 0.5—1.0 mg of protein in 0.1—0.3 ml of water in an Intertechnique Scintillation counter. The scintillation fluid contained: dioxane 100 ml, PPO 400 mg, POPOP 20 mg, naphtalene 20.8 g, methanol 60 ml and toluene 100 ml. Autoradiography was performed by using X-ray film (Wicor X-RP).

RESULTS AND DISCUSSION

From the brain and liver ribosomes of 14-day old chick embryos, 0.3 and 0.8 mg protein per 1 g fresh tissue weight were obtained, respectively. This value was similar to that reported by Ashby and Roberts (1).

Figure 1 presents a typical profile of resolution of the brain and liver ribosomal proteins on the CM-cellulose column. According to the applied stepwise gradient (0.01 and 0.02 N HCl) two fractions designated as f_1 and f_2 were obtained. The mean percent contents of f_1 and f_2 were 40 and 60, respectively. Polyacrylamide-gel electrophoresis patterns of the proteins isolated from the brain and liver ribosomal preparations, and theirs fractions, showed several significant differences (Fig. 2). Both fractions differ from one another. As one can see, fraction f_1 contains mostly the low molecular weight and rather basic proteins, whereas in fraction f_2 predominated the high molecular weight and acidic ones. Moreover, the used technique allowed to visualize differences between the brain and liver ribosomal proteins, which were slightly visible on the one-dimensional polyacrylamide-gel electrophoresis.

Some workers have not found any differences between ribosomal proteins of separate organs in a single animal (13), but MacInnes (12) has observed some quantitative differences between the brain and liver ribosomal proteins.

All fractions (Table 1) of ribosomal proteins from the brain and the liver were found to contain phosphate. The fractions did not contain any inorganic or nucleotide phosphates as shown in the previous work (19) and the phosphate was present in phosphoserine and phosphothreonine as described previously (18). The contents of phosphate in the total ribosomal proteins and in the particular fractions from the brain were

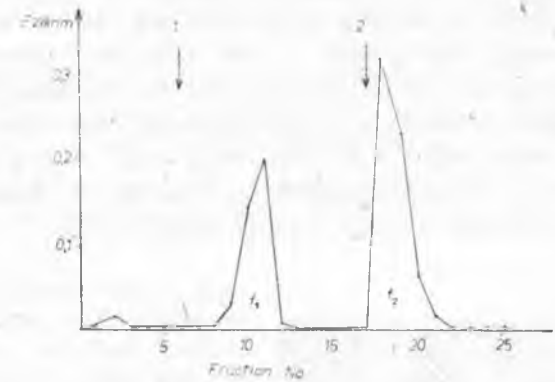

Fig. 1. CM-cellulose column chromatography of ribosomal proteins. Protein, 2—3 mg, in acetate buffer pH=4.2 was applied to the column (0.9×6 cm) and eluted successively with: 1 — 0.01 N HCl, 2 — 0.02 N HCl. Fractions of 9 ml were collected and extinction at 278 nm was determined

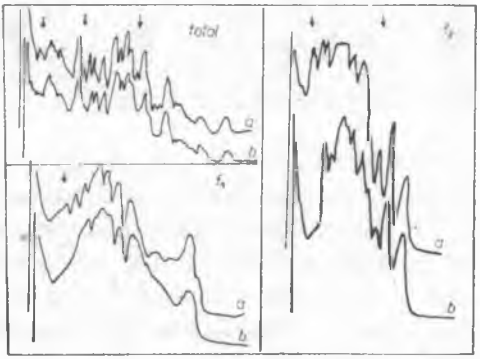

Fig. 2. Densitometric tracing of amido black stained acrylamide gels containing total protein and their fractions (f_1, f_2) from the brain (a) and the liver (b) ribosomes. The arrows indicate the differences

somewhat higher than those from the liver. It may be of interest to add that fraction f_2 had three times more of phosphorus than fraction f_1, in both tissues.

Systematic investigations of the radioactive phosphorus incorporation were performed by the determination of its specific activity in the par-

Table 1. The content of phosphate in the brain and liver ribosomal proteins from chick embryos (μg/mg protein)

Fraction	Brain	Liver
Total protein	2.5 ±0.15	1.6 ±0.10
f_1 — eluted with 0.01 N Cl	0.9 ±0.05	0.5 ±0.05
f_2 — eluted with 0.02 N HCl	2.7 ±0.14	1.7 ±0.08

The results are mean values of 4—5 determinations ±SD.

ticular fractions after 4, 10 and 20 h following isotope administration. The label of phosphorus detected in the ribosomal proteins made up only a small percentage of the phosphorus incorporated into the ribosomes. The highest percentage of radioactivity was observed in 10 h experiments; it was 2 and 3% for the brain and the liver, respectively. The radioactivity of ^{32}P corresponded to the spots of phosphoserine and phosphothreonine as indicated by autoradiography (Fig. 3).

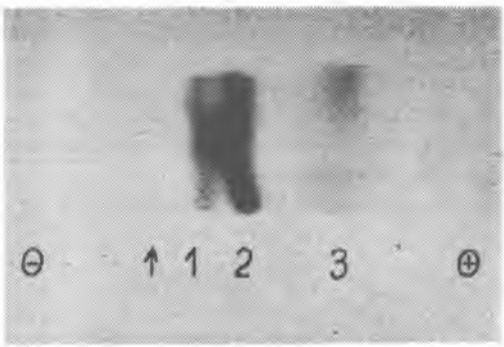

Fig. 3. Autoradiograph of paper electrophoregram of acid hydrolysate of ^{32}P-labelled ribosomal proteins. Protein was hydrolyzed in 3 N HCl for 4 h at 105°C. Electrophoresis was carried out in formic—acetic acid buffer $pH = 2.2$ for 60 min., at 20 v/cm. Autoradiograph was exposed for 5 days. The radioactive strips corresponded to those of pattern samples of phosphothreonine (1), phosphoserine (2) and inorganic phosphate (3) which run in parallel. The arrow indicates the origin

The kinetics of incorporation of ^{32}P into the unfractionated ribosomal proteins was similar in both tissues, although the rate and the extent of ^{32}P incorporation were different in both cases. In all the experiments, the specific activity of the ribosomal proteins in the liver was higher than that of the ribosomal proteins in the brain. The differences were the highest in short-time experiments and the smallest ones in the 20 h experiments (Fig. 4A).

The kinetics of phosphorylation of fraction f_1 was different in both tissues. In the liver the highest specific activity was observed after 4 h and it was twice as high as that in the brain, fraction f_1. The latter reached maximum after 10 h. In 20 h experiments both fractions had the same specific activity (Fig. 4B).

On the other hand, the profile of phosphorylation of f_2 was similar in the brain and the liver. In both tissues the incorporation of phosphate increased with time, but the increase was unequal. The increase after 10 h was 4-fold in the brain, and only 2-fold in the liver of that after 4 h. The difference between 10 and 20 h was negligible (Fig. 4C).

Since the particular protein fractions differ in the content of phosphorus, the radioactivity was calculated also per 1 μg P. All the differences between both these tissues were significantly higher (Fig. 4D, E, F).

The kinetics studies indicated that incorporation of ^{32}P into the liver

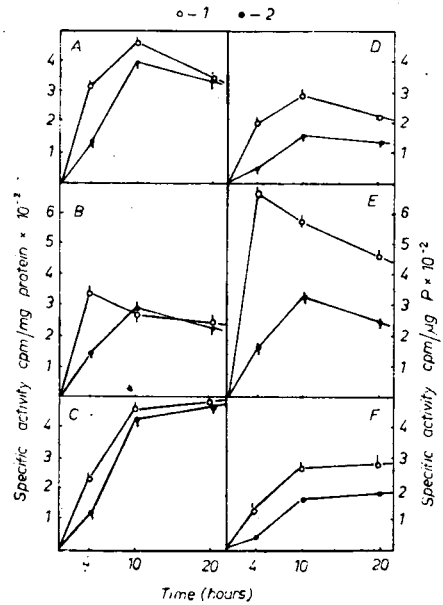

Fig. 4. Kinetics of incorporation of radioactivity from (^{32}P) — orthophosphate into total protein (A, D) and their fractions: f_1 (B, E) and f_2 (C, F) from the liver (1) and the brain (2) ribosomes. Each point represents the mean ±SD from 4—5 experiments

ribosomal proteins, was much rapid and slightly higher, than that into the brain ribosomal proteins. These differences were not related to the methods used to isolate ribosomal proteins and to the content of phosphate. It may be assumed that labelling intensity is due to a different mitotic activity of these tissues.

The differences in the time-course of phosphate incorporation into fractions f_1 and f_2 point to some distinct differences in the rate of phosphate turnover. The higher rate was observed for fraction f_1 in both tissues. These observations are in agreement with the previous findings on the ^{14}C-lysine incorporation (16).

REFERENCES

1. Ashby C. D., Roberts S.: Phosphorylation of Ribosomal Proteins in Cerebral Cortex *in vitro*. J. Biol. Chem. **250**, 2546, 1975.
2. Barden N., Labrie F.: Cyclic Adenosine 3.4-monophosphate-dependent Phosphorylation of Ribosomal Proteins from Bovine Anterior Gland. Biochemistry **12**, 3096, 1973.
3. Bitte L., Kabat D.: Phosphorylation of Ribosomal Proteins in Sarcoma 180 Tumor Cells. J. Biol. Chem. **247**, 5345, 1972.
4. Blat C., Loeb J. E.: Effect of Glucagon on Phosphorylation of some Rat Liver Ribosomal Proteins *in vivo*. Fed. Eur. Biochem. Soc. Letters **18**, 124, 1971.
5. Correze C. et al.: Effect of Thyroid Hormones on Phosphorylation of Liver Ribosomal Proteins and on Protein Phosphokinase Activity. Fed. Eur. Biochem. Soc. Letters **23**, 87, 1972.

6. Gressner A. M., Wool I. G.: The Phosphorylation of Liver Ribosomal Proteins *in vivo*. Evidence that only a Single Small Subunit Protein (S_6) is Phosphorylated. J. Biol. Chem. **249**, 6917, 1974.
7. Hurst R. O.: Determination of Nucleotidde Phosphorus with a Stannous Chloride Hydrazine Sulfate Reagent. Can. J. Biochem. **42**, 287, 1964.
8. Johns E. W.: The Electrophoresis of Histones in Polyacrylamide Gel and their Quantitative Determination. Biochem. J. **104**, 78, 1967.
9. Kabat D.: Phosphorylation of Ribosomal Proteins in Rabbit Reticulocytes. Characterization and Regulatory Aspects. Biochemistry **9**, 4160, 1970.
10. Leader D. P. et al.: The Phosphorylation of Ribosomal Protein S_6 in Baby Hamster Kidney Fibroblasts. Biochem. Biophys. Res. Comm. **71**, 966, 1976.
11. Lowry O. H. et al.: Protein Measurement with Folin-reagent. J. Biol. Chem. **193**, 265, 1951.
12. MacInnes J. W.: Difference between Ribosomal Subunits from Brain and those from other Tissues. J. Mol. Biol. **65**, 157, 1972.
13. Mutolo V. et al.: Species Specifity of Embryonic Ribosomal Proteins. Biochim. Biophys. Acta **138**, 214, 1967.
14. Roberts S., Morelos B. S.: Phosphorylation of Multiple Proteins of both Ribosomal Subunits in Rat Cerebral Cortex *in vivo*. Effect of Adenosine 3 : 5 — cyclic monophosphate. Biochem. J. **184**, 233, 1979.
15. Rupp R. et al.: Phosphorylation of Ribosome-associated Proteins during the Mammalian Cell Cycle. Unique Phosporylation of a Specific Protein during Mitosis. Biochim. Biophys. Acta **418**, 81, 1976.
16. Sanecka-Obacz M., Borkowski T.: Incorporation of ^{14}C-lysine into Basic Proteins of the Brain Ribosomes. Bull. Acad. Polon. Sci., Ser. Sci. Biol. **21**, 709, 1973.
17. Sanecka-Obacz M.: Fosforylacja zasadowych białek rybosomalnych w embriogenezie. XI Zjazd Pols. Biochem. Stow., Streszczenia E-3, Białystok 1973, p. 164.
18. Sanecka-Obacz M. et al.: Prosta metoda wykrywania fosfoseryny i fosfotreniny w białkach. Ann. Univ. M. Curie-Skłodowska, Lublin **34**, 47, 1979.
19. Sanecka-Obacz M., Borkowski T.: Phosphorylation of Protein from Rabbit Ribosomes. Acta Biochim. Pol. **21**, 397, 1974.

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STRESZCZENIE

Zarodkom kurzym 14-dniowym podawano doowodniowo ^{32}P-ortofosforan. Po 4-, 10- i 20-godzinnej inkubacji oznaczano w mózgu i wątrobie aktywność specyficzną białek rybosomalnych oraz ich frakcji, uzyskanych po rozdziale na kolumnie z CM-celulozy.

Inkorporacja ^{32}P-ortofosforanu do białek rybosomów wątroby przebiegała szybciej i nieznacznie intensywniej niż do białek rybosomów mózgowych. W obu tkankach frakcja f_1 wykazywała żywszy *turnover* ^{32}P niż frakcja f_2.

РЕЗЮМЕ

Куриные 14-дневные эмбрионы получали в амниотический мешок меченый фосфор. Спустя 4, 10 и 20 часов инкубации, определяли в мозге и печени радиоактивность рибосомальных белков, а также их фракции, полученные после фракционирования на колонке СМ-целлюлозы.

Инкорпорация ^{32}P-ортофосфата в рибосомальные белки печени происходила быстрее и несколько интенсивнее, чем в рибосомальные белки мозга. В обоих тканьях фракция $Ф_1$ проявляла более быстрый обмен ^{32}P, чем фракция $Ф_2$.