Vol. 102, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1981 Pages 568-573

IN VITRO SYNTHESIS OF A PUTATIVE PRECURSOR OF SERINE:PYRUVATE AMINOTRANSFERASE OF RAT LIVER MITOCHONDRIA

Toshiaki Oda, Arata Ichiyama, Satoshi Miura*, Masataka Mori*, and Masamiti Tatibana*

Department of Biochemistry, Hamamatsu University School of Medicine, Handa-cho, Hamamatsu, Shizuoka 431-31, Japan
*Department of Biochemistry, Chiba University School of Medicine, Inohana, Chiba 280, Japan

Received August 10,1981

SUMMARY

Serine:pyruvate aminotransferase [EC 2.6.1.51] of rat liver, an enzyme induced by glucagon in mitochondria, was synthesized in cell-free protein synthesizing systems derived from nuclease-treated rabbit reticulocyte lysate and wheat germ extract as a putative precursor which was approximately 2,000 daltons larger than the subunit of mature enzyme. The hepatic level of translatable messenger RNA coding for the putative precursor was approximately 40 times higher in rats received a glucagon administration 3.5 h before sacrifice than in control animals.

In the liver of normal fed and fasted rats, the activity of serine:pyruvate aminotransferase is detected in peroxisomes, mitochondria, and cytosolic fraction in a roughly equal ratio (1)[1] but only the mitochondrial activity is remarkably increased after administration of glucagon (1,2). The physicochemical (3), kinetic (3), and immunological[1] properties of the mitochondrial and peroxisomal enzymes were indistinguishable from each other. This enzyme has been shown to consist of two identical subunits of approximate Mr=40,000 (2-4). We have previously shown that the increased activity after glucagon injection is accompanied by the parallel increase in the amount of the enzyme protein and that the accumulation of the enzyme can be ascribed mainly to the rise in the rate of enzyme synthesis (2).

Abbreviations: pSPT, precurosr of serine:pyruvate aminotransferase; SDS, sodium dodecyl sulfate; anti-SPT serum, anti-serine:pyruvate aminotransferase serum.

[1] Oda, T., Yanagisawa, M., and Ichiyama, A., submitted for publication

0006-291X/81/170568-06$01.00/0

We have also presented evidence showing that ornithine carbamoyltransferase [EC 2.1.3.3], a hepatic mitochondrial matrix enzyme in ureotelic animals, is initially synthesized on cytoplasmic membrane-free ribosomes as an approximately 3,400 daltons larger precursor, which is then transported into mitochondrial matrix in association with post-translational processing to make the mature form of the enzyme (5-7). Other mitochondrial matrix proteins including carbamoyl phosphate synthetase I [EC 6.4.3.16] (8,9), δ-aminolevulinate synthetase [EC 2.3.1.37] (10), aspartate:α-ketoglutarate aminotransferase [EC 2.6.1.1] (11,12), and adrenodoxin (13) have also been shown to be synthesized as larger precursors.

In this communication we present evidence indicating that serine:pyruvate aminotransferase of rat liver is synthesized initially as a precursor, which is approximately 2,000 daltons larger than the mature subunit, and that the level of translatable mRNA coding for the precursor is greatly increased by glucagon administration.

MATERIALS AND METHODS

Animals ----- Male rats of Wistar strain (140-160 g) were fed a standard laboratory chow and were fasted for 24 h before use. Glucagon was freshly dissolved in 5 mM NaOH and injected intraperitoneally into the fasted rats at a dose of 300 μg/100 g body weight 3.5 h and 24 h prior to sacrifice.

Preparation of antibody ----- Serine:pyruvate aminotransferase was purified to homogeneity from mitochondria of glucagon-injected rats essentially as described previously (2). Anti-SPT serum was obtained from a rabbit which had been immunized with the purified enzyme as reported previously (2).

Cell-free protein synthesis ----- Total RNA was isolated from the livers of control and glucagon-treated rats by the SDS-phenol method as described previously (9). Nuclease-treated rabbit reticulocyte lysate and wheat germ extract were prepared as described by Pelham and Jackson (14) and Nakanishi et al. (15), respectively. Cell-free protein synthesis was carried out as described previously (9) except that concentrations of RNA, K-acetate, Mg-acetate, spermidine, and creatine phosphokinase in the assay mixture were 1.2 mg/ml, 120 mM, 0.8 mM, 0.6 mM, and 44 μg/ml, respectively, and KCl was omitted. The concentration of RNA was estimated using an extinction coefficient of $E^{1\%}_{260\ nm}$ =200. Immunoreaction of the cell-free translation product with anti-SPT serum followed by adsorption to protein A-bearing Staphylococcus aureus (16), extraction (16), SDS-12% polyacrylamide slab gel electrophoresis (17), and fluorography of the dried gels (18) were carried out by the cited methods. The radioactive protein bands on the diphenyloxazole-impregnated dried gels were cut out and counted for radioactivity in a toluene scintillant with an efficiency of about 77% (6). Strips of the same size were cut out from a region just below the protein bands with no visible grains, and used as background of the radioactivity determination.

Labeling of mature serine:pyruvate aminotransferase in isolated hepatocytes ----- Isolated rat hepatocytes (2.5 x 10^7 cells/ml) were incubated with [^{35}S]methionine (300 μCi/ml) for 40 min at 37°C as described previously (19). Particulate components containing mitochondria were prepared and solubilized in the buffer containing 0.1% SDS and 0.1% Triton X-100 (19). The labeled mature aminotransferase was isolated by immunoprecipitation with anti-SPT serum and S. aureus cells.

Materials ----- [^{35}S]Methionine (>800 Ci/mmol) was obtained from New England Nuclear, microbial protease inhibitors (antipain, leupeptin, chymostatin, and pepstatin) were from the Peptide Institute (Osaka, Japan), glucagon was from Sigma, S. aureus nuclease was from Boehringer Mannheim GmbH, and X-ray films (Fuji-Rx) were from Fuji Film (Tokyo, Japan).

RESULTS AND DISCUSSION

Figure 1 shows the results of the cell-free translation of serine:pyruvate aminotransferase. When total RNA isolated from rat livers was used to direct protein synthesis in the nuclease-treated rabbit reticulocyte lysate system, incorporation of [^{35}S]methionine into total (trichloroacetic acid-insoluble) translation product was increased approximately 15-fold as compared with that in the absence of the exogenous RNA. A large number of different proteins including those of larger molecular weight were synthesized under these conditions (A, lanes 1-3), indicating that hepatic mRNA's of both the fasted and glucagon-treated rats were obtained without suffering serious ribonuclease attack. When immunoprecipitable translation product was analyzed by SDS-polyacrylamide gel electrophoresis and fluorography, a single major band was detected (A, lanes 5 and 6). Total RNA from rats injected with glucagon 3.5 h prior to sacrifice was apparently more active in directing the synthesis of the polypeptide than the RNA from rats that received the hormone 24 h before (A, lanes 5 and 6). RNA from fasted rats was almost devoid of this activity (A, lane 4). Beside the major band, several minor bands were observed. These bands were also seen when the antibody was replaced by a control rabbit serum (data not shown), suggesting that they were derived from non-specific adsorption. The molecular weight of the major band was approximately 2,000 larger than that of the mature serine:pyruvate aminotransferase labeled with [^{35}S]methionine in isolated hepatocytes (A, lane 9) and the purified enzyme (molecular weight of subunit: 40,000). Immunological identity between the in vitro product and serine:pyruvate

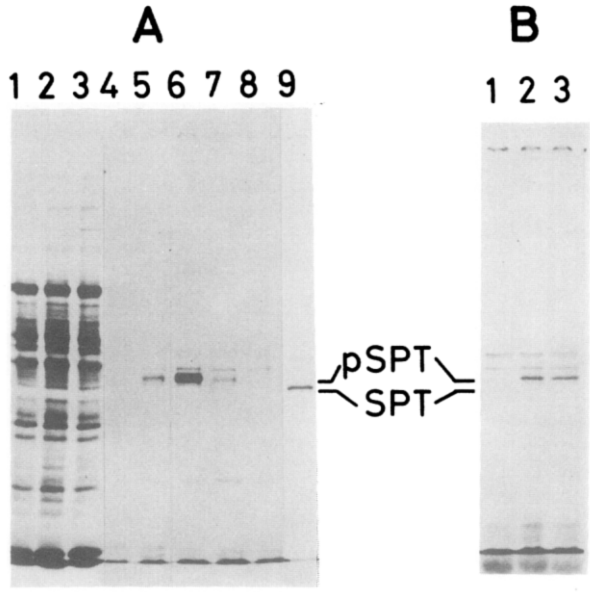

Fig. 1. Total hepatic RNA-directed in vitro synthesis of pSPT by rabbit reticulocyte lysate (A) and wheat germ extract (B).

Cell-free translation was performed with total hepatic RNA isolated from 24 h-fasted rats (A, lanes 1 and 4, B, lane 1) and the animals injected with glucagon 24 h (A, lanes 2 and 5, B, lane 2) or 3.5 h (A, lanes 3 and 6-8, B, lane 3) prior to sacrifice. The RNA isolated from two rats each was combined and used. Total translation product (A, lanes 1-3) and immunoprecipitable product recovered with 10 µl of anti-SPT serum in the absence (A, lanes 4-6) or presence of 10 µg and 50 µg of purified serine:pyruvate aminotransferase (A, lanes 7 and 8, respectively) were analyzed on SDS-12% polyacrylamide gel electrophoresis followed by fluorography. Mature serine:pyruvate aminotransferase labeled with [^{35}S]methionine in isolated hepatocytes as described under "MATERIALS AND METHODS" was similarly immunoprecipitated and subjected to the electrophoresis (A, lane 9). Gel sheets were exposed to X-ray films at -80°C for 2.5 days (A, lanes 1-8), 5 days (A, lane 9), and 7 days (B, lanes 1-3), respectively.

aminotransferase was demonstrated by the fact that excess unlabeled mature enzyme competed effectively with the in vitro product for interaction with the antibody (A, lanes 7 and 8). These data thus suggest that the polypeptide observed in lanes 5 and 6 of Fig. 1-A is a precursor of serine:pyruvate aminotransferase (pSPT) and that glucagon causes a marked increase in the level of functional mRNA coding for pSPT. The putative precursor was also synthesized when wheat germ extract was used instead of rabbit reticulocyte lysate as the protein synthesizing system (Fig. 1-B).

The effect of glucagon on the level of mRNA coding for pSPT was assessed by counting the radioactivity of the protein band cut out from the dried gels

Table I. RNA-directed incorporation of [^{35}S]methionine into pSPT and the effect of glucagon administration

For determination of the radioactivity incorporated into pSPT, the pSPT-bands of lanes 4-6 of Fig. 1-A were cut out and the radioactivity was counted as described under "MATERIALS AND METHODS." Radioactivity incorporated into total translation product was determined as described previously (16).

RNA from livers of rats	Incorporation of [^{35}S]methionine into				pSPT / total protein
	total protein		pSPT		
	cpm	(ratio)	cpm	(ratio)	%
fasted for 24 h	925,300	(1)	31	(1)	0.003
Glucagon, 3.5 h before	1,448,000	(1.6)	1,303	(42)	0.090
Glucagon, 24 h before	1,376,000	(1.5)	309	(10)	0.022

(Table I). The activity of RNA to direct the incorporation of [^{35}S]methionine into pSPT was increased as much as 42 times and 10 times by the injection of glucagon 3.5 h and 24 h before sacrifice, respectively. The incorporation of the labeled amino acid into total translation product was, on the other hand, only slightly affected by the hormone treatments. The synthesis of pSPT directed by the total RNA from rats fasted for 24 h and from the animals injected with glucagon 3.5 h prior to sacrifice accounted for approximately 0.003% and 0.09% of the total protein synthesis, respectively (Table I).

It has been shown that serine:pyruvate aminotransferase in rat liver mitochondria is remarkably increased by the administration of glucagon (1,2).[1] In a preliminary experiment we have observed that the enzyme is synthesized in response to the glucagon stimuli on extramitochondrial free ribosomes (unpublished data). These observations and the drastic increase of the level of mRNA coding for pSPT by glucagon presented in this paper suggest that mitochondrial serine:pyruvate aminotransferase is synthesized as the precursor on cytoplasmic ribosomes and transported into mitochondria in association with

processing to the mature enzyme. The results also indicate that glucagon brings about the induction of the enzyme, at least in part, by stimulating synthesis of or by inhibiting degradation of mRNA for pSPT or both.

ACKNOWLEDGMENTS

This study was supported in part by Grant-in-Aids for Scientific Research (1978-1980) and for Encouragement of Young Scientist (1980) from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Noguchi, T., Minatogawa, Y., Takada, Y., Okuno, E., and Kido, R. (1978) Biochem. J. 170, 173-175
2. Fukushima, M., Aihara, Y., and Ichiyama, A. (1978) J. Biol. Chem. 253, 1187-1194
3. Noguchi, T., and Takada, Y. (1978) Biochem. J. 175, 765-768
4. Noguchi, T., Okuno, E., and Kido, R. (1976) Biochem. J. 159, 607-613
5. Mori, M., Miura, S., Tatibana, M., and Cohen, P.P. (1980) J. Biochem. (Tokyo) 88, 1829-1836
6. Mori, M., Miura, S., Tatibana, M., and Cohen, P.P. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 7044-7048
7. Morita, T., Mori, M., Tatibana, M., and Cohen, P.P. (1981) Biochem. Biophys. Res. Commun. 99, 623-629
8. Shore, G.C., Carignan, P., and Raymond, Y. (1979) J. Biol. Chem. 254, 3141-3144
9. Mori, M., Miura, S., Tatibana, M., and Cohen, P.P. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5071-5075
10. Yamauchi, K., Hayashi, N., and Kikuchi, G. (1980) FEBS Lett. 115, 15-18
11. Sonderegger, P., Jaussi, R., and Christen, P. (1980) Biochem. Biophys. Res. Commun. 94, 1256-1260
12. Sakakibara, R., Huynh, Q.K., Nishida, Y., Watanabe, T., and Wada, H. (1980) Biochem. Biophys. Res. Commun. 95, 1781-1788
13. Nabi, N., and Omura, T. (1980) Biochem. Biophys. Res. Commun. 97, 680-686
14. Pelham, H.R.B., and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247-256
15. Nakanishi, S., Taii, S., Hirata, Y., Matsukura, S., Imura, H., and Numa, S. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4319-4323
16. Mori, M., Miura, S., Tatibana, M., and Cohen, P.P. (1981) J. Biol. Chem. 256, 4127-4131
17. Laemmli, U.K. (1970) Nature 227, 680-685
18. Bonner, W.M., and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83-88
19. Mori, M., Morita, T., Ikeda, F., Amaya, Y., Tatibana, M., and Cohen, P.P. (1981) Proc. Natl. Acad. Sci. U.S.A., in press