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SYNTHESIS OF GLUTAMIC OXALOACETIC TRANSAMINASE ISOZYMES IN RAT LIVER CELLS

Ryuzo Sakakibara, Motohiko Takemura, Yoshinori Kamisaki, Yoshiyuki Horio and Hiroshi Wada

Departments of Pharmacology II , Osaka University Medical School, Kita-ku, Osaka, 530 Japan

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The synthesis of glutamic oxaloacetic transaminase isozymes in rat liver explants was studied using specific antisera against the cytosolic and mitochondrial isozymes. The pulse-labeled cytosolic isozyme was detected in the cytosolic fraction and remained there in pulse-chase experiments. On the other hand, the pulse-labeled mitochondrial isozyme was detected as a larger precursor in the cytosolic fraction. During chase, the amount of pulse-labeled precursor of the mitochondrial isozyme decreased and labeled mature mitochondrial isozyme appeared in the mitochondrial fraction.

Glutamic oxaloacetic transaminase exists as two distinct enzymes in higher organisms (1), one located in the cytoplasm (sGOT) (2), and the other in the mitochondria (mGOT)(3). Both isozymes are coded by nuclear genomes and are synthesized on cytoplasmic ribosomes (4,5).

We previously found that GOT isozymes were synthesized in vitro (in a cell-free protein synthesizing system of rabbit reticulocyte lysate) as putative precursors. Precursor of sGOT from rat liver synthesized in vitro had a similar molecular weight to the subunit of its mature form (5). On the other hand, mGOT precursors (p-mGOT) from rat liver (5) and chicken heart (4) synthesized in vitro had molecular weights of about 2,000 and 3,000 daltons, respectively, more than the mature subunit. We further found that

Abbreviations used : GOT, glutamic oxaloacetic transaminase [L-aspartate : 2-oxoglutarate aminotransferase (EC 2.6.1.1.)] ; sGOT and mGOT, cytoplasmic and mitochondrial isozyme, respectively ; p-sGOT and p-mGOT, a putative precursor of sGOT and mGOT ; SDS, sodium dodecyl sulfate ; SDS-PAGE, sodium docecyl sulfate-polyacrylamide gel electrophoresis.

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p-mGOT was translocated into the mitochondria in association with processing of p-mGOT to its mature form when incubated with freshly isolated rat liver mitochondria (6).

These in vitro experiments suggest that rat liver mGOT may be synthesized as a larger precursor in the cytosol and then transported into mitochondria like other mitochondrial enzymes (7-17). In this paper, we report that p-mGOT can be detected in the cytosol of rat liver cells and is then translocated into mitochondria, while sGOT remains in the cytosol.

MATERIALS AND METHODS

Liver Explants: The liver from a decapitated male Sprague Dawley rat (BW=270g) was sliced into about 0.5 mm thick sections with a razor. The explants (each about 50 mg wet weight) were washed with phosphate buffered saline and incubated for various times with shaking in a water bath at 37°C under air in scintillation vials in 1 ml of medium containing 65 mM KCl, 3.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 71 mM NaCl, 6.2 mM sodium phosphate (pH 7.6), and 19 amino acids (0.1 mM each) other than methionine as described by Morita et al. (9). The preparation was labeled with [^{35}S] methionine (100 μCi/ml, 1000 Ci/mmole, New England Nuclear) for various times at 37°C (pulse-labeling). Pulse-labeling and chase experiments were followed by further incubation for 30 min in the same medium except that unlabeled methionine (0.1 mM) was used instead of labeled methionine.

Subcellar Fractionation of Rat Liver Explants : After pulse-labeling or pulse-labeling and chase, slices were cooled to 4°C and homogenized at 4°C with a Potter-type microhomogenizer (4 times up and down) in 0.5 ml of 10 mM HEPES buffer (pH 7.4) containing 100 mM KCl, 250 mM sucrose, and 0.7 mg/ml phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 8,000 x g (10 min) at 4°C to precipitate the mitochondria and the supernatant was centrifuged at 105,000 x g (60 min) to obtain the cytosol fraction. The crude mitochondrial fraction was lysed with 1 ml of 10 mM HEPES buffer (pH 7.5), 0.5 % Triton X-100, 0.7 mg/ml phenylmethylsulfonyl fluoride and then centrifuged at 105,000 x g (60 min) to remove insoluble material. Aliquots ($\frac{1}{100}$ vol) of the resulting mitocondrial supernatant and cytosolic fraction were used for assay of trichloroacetic acid (TCA)-insoluble radioactivity as described (18) and 0.25 ml ($\frac{1}{4}$ vol) of crude mitochondrial supernatant and the whole cytosolic fraction were analyzed by immunoprecipitation.

Immunoprecipitation : Immunoprecipitation was performed as described in (5) using specific antisera (50 μl) against rat mGOT and sGOT, respectively, and fixed Staphylococcus aureus cells (100 μl of 10 % suspension), in 0.5 ml of 50 mM Tris-HCl (PH 7.4), containing 150 mM NaCl, 1 % Triton-X 100, 0.1 % SDS, 10 mM methionine, 5 mM EDTA, and 150 μg/ml phenylmethylsulfonyl fluoride. The cells were washed three times with 2 ml of the same buffer, and then extracted for 3 min at 100°C with 30 μl of SDS-PAGE sample buffer (150 mM Tris-HCl, pH 6.8, 5 % SDS, 5 % 2-mercaptoethanol, 10 % glycerol, 0.001 % bromophenol blue). Cells were removed by brief centrifugation and the supernatant was analyzed by SDS-polyacrylamide (10 %) gel electrophoresis (19) and fluorography (20).

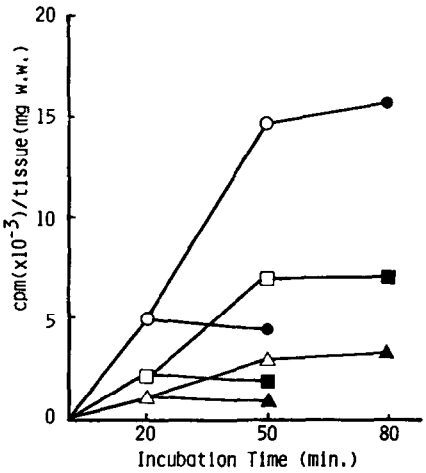

Fig 1. Pulse and pulse-chase kinetics of incorporation of [^{35}S] methionine into rat liver explants.
Rat liver explants (each 50 mg wet weight) were incubated with [^{35}S] methionine for 0, 20 and 50 min at 37°C. One culture each was chased with unlabeled methionine for 30 min at 20 and 50 min, respectively, after incubation with [^{35}S] methionine. At the times indicated, samples were homogenized, subcellular fractions were seperated and [^{35}S] radioactivity in protein in each fraction was determined.
○ , homogenate ; □ , crude mitochondrial fraction ; △ , cytosolic fraction ; open symbols, pulse-labeling ; closed symbols, pulse-labeling and chase.

Miscellaneous
Purification of rat liver GOT isozymes, preparation of antisera against rat liver GOT isozymes and preparation of tritium labelled GOT isozymes were performed as described previously (5, 21). Protein was determined by the method of Lowry et al. (22) with bovine serum albumin as a standard.
Chemicals : All other chemicals used were standard commercial products.

RESULTS AND DISCUSSION

As shown in Fig. 1, when rat liver explants were incubated with [^{35}S] methionine, the amount of pulse labeled proteins increased time-dependently. After pulse-labeling, chasing for 30 min with unlabeled methionine stopped the increase in radioactivity at a level similar to that before chase.

Synthesis of sGOT in Rat Liver Explants : When rat liver explants were incubated with [^{35}S]-methionine (pulse-labeling) and then their subcellular fractions were examined, pulse-labeled sGOT was detected after 20 min exclusively in the cytosolic fraction (Fig 2A) with none in the crude mitochondrial fraction (Fig 2B). The amount of

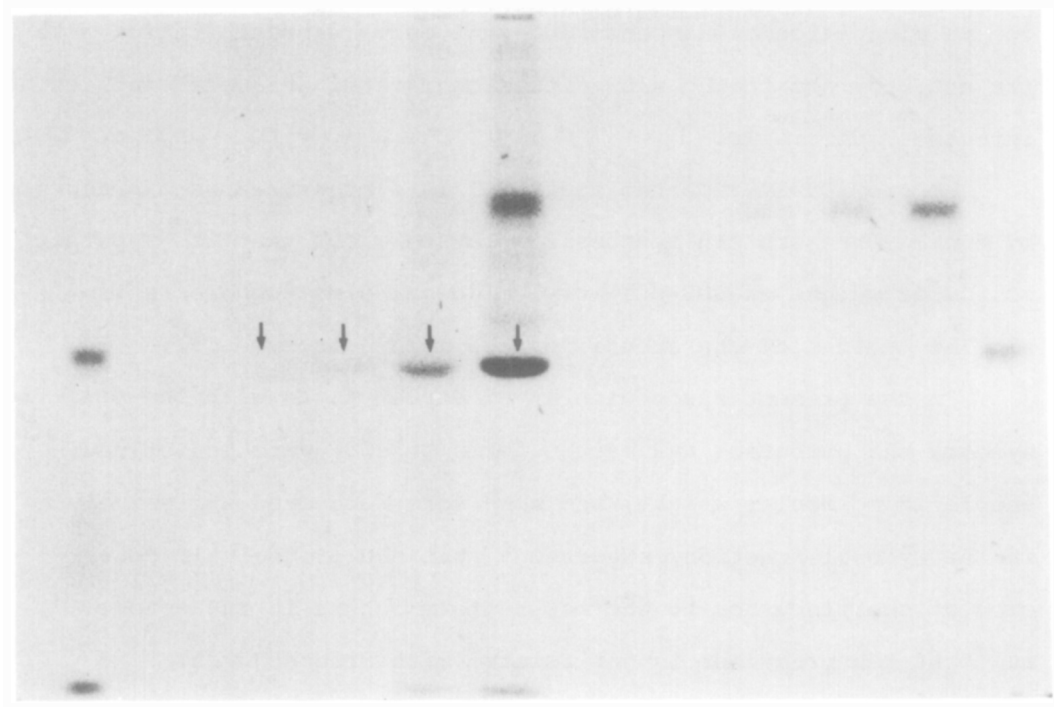

Fig 2. Pulse-labeled sGOT detected in the cytosol of rat liver explants.
Five rat liver explants were pulse-labeled with [^{35}S] methionine for 0 (lane 1), 20 (lanes 2 and 3) and 50 min (lanes 4 and 5) at 37°C. Then, one liver explant each was chased for 30 min after pusle-labeling for 20 min (lane 3) and 50 min (lane 5), respectively. Explants were homogenized, and subcellular fractions were separated and subjected to immunoprecipitation using anti-sGOT antiserum, and to SDS-PAGE and fluorography as described in the MATERIALS AND METHODS.
(A), cytosolic fraction ; (B), crude mitochondrial fraction ; p-sGOT, sGOT synthesized *in vitro* (5) ; sGOT, tritium labeled sGOT (5). Downward arrows indicate bands corresponding to sGOT and p-sGOT.

pulse-labeled sGOT was increased with time (Fig 2A, lanes 2 and 4). The apparent molecular weight of pulse-labeled sGOT was similar to that of the subunit of its mature form, like that of sGOT synthesized *in vitro* (5). When rat liver explants were incubated for 20 and 50 min with [^{35}S]-methionine and subsequently chased for 30 min in the presence of unlabeled methionine, the amount of labeled sGOT was larger (Fig 2-A, lanes 3 and 5) than the amount found without chase (Fig 2A, lanes 2 and 4). This was probably

due to elongation of nascent sGOT that escaped precipitation with its antibody and to the slow diffusion rate of unlabeled methionine into the liver cells.

We previously reported that sGOT is synthesized as a precursor by a cell-free protein synthesizing system, but that its apparent molecular weight on SDS-polyacrylamide gel electrophoresis is similar to that of the subunit of the mature isozyme (5).

In the present study with liver explants, as with the cell-free system, the precursor and mature forms of sGOT were indistinguishable in molecular weight, and both were recovered exclusively in the cytosol fraction, suggesting that the proteolytic conversion of the precursor to the mature form occurs in the cytosol, and that the precursor is not related with mitochondria.

Synthesis of mGOT in Rat Liver Explants : As shown in Fig 3B (lane 4), when rat liver explants were pulse-labeled with [^{35}S]-methionine and then their subcellular fractions were examined, pulse-labeled mGOT was detected at 50 min in the mitochondrial fraction. The apparent molecular weight of pulse-labeled mGOT detected in the mitochondrial fraction was identical to that of the subunit of mature mGOT. The cytosolic fraction contained two kinds of labeled mGOT (Fig 3-A, lane 4) that both competed with cold mature mGOT in immunoprecipitation (data not shown). Material in the band indicated by an upward arrow in Fig 3-A had the same molecular weight as the mature enzyme and mGOT found in the crude mitochondrial fraction ; material in the other band, indicated by a downward arrow, was p-mGOT synthesized in vitro.

On the other hand, p-mGOT pulse-labeled for 20 min was found only in the cytosolic fraction (Fig 3A, lane 2). With our procedure of subcellular fractionation of liver explants, about 10 % of the endogenous mGOT was detected by gel electrophoresis in the cytosolic fraction (data not shown). However, judging from these

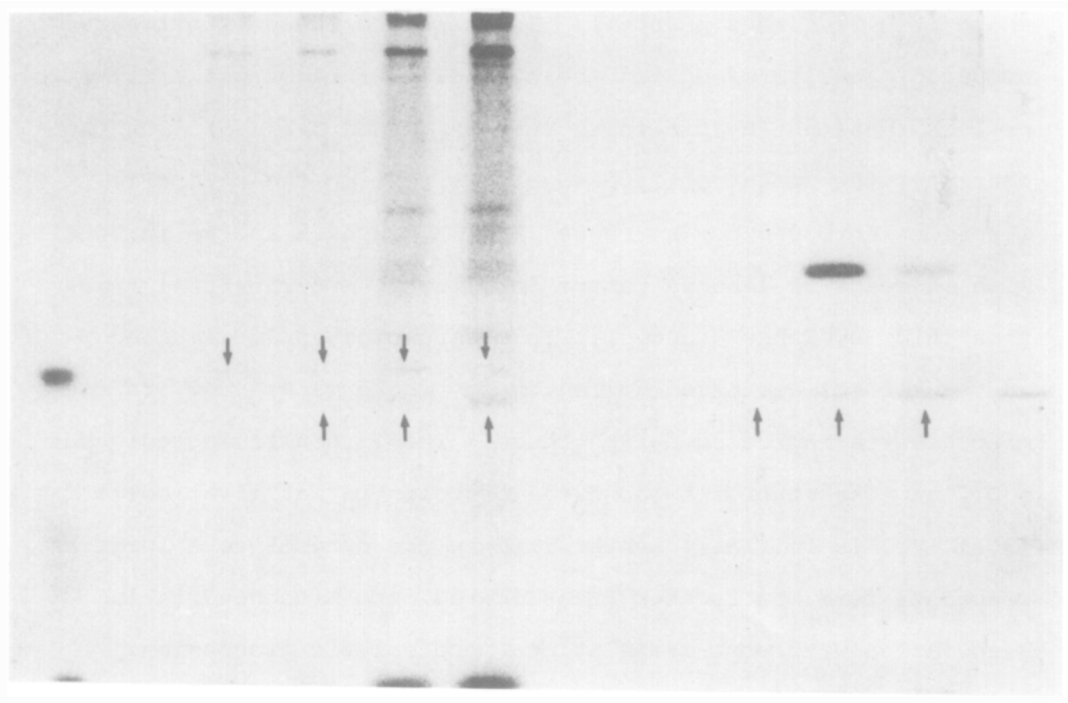

Fig 3. Pulse-labeled p-mGOT was detected in the cytosol of rat liver explants.
Five rat liver explants were pulse-labeled and pulse-chased, and homogenized and subcellular fractions were obtained as outlined in the legend to Fig 2. Immunoprecipitation were carried out with anti-mGOT antiserum.
(A) cytosolic fraction ; (B), crude mitochondrial fraction ; p-mGOT, mGOT synthesized *in vitro* (5) ; mGOT, tritium labeled mGOT (5). Downward and upward arrows indicate bands coresponding to p-mGOT and mGOT, respectively.

results, this labeled mature mGOT in the cytosol had probably been released from mitochondria during cell fractionation. It is concluded , therefore, that radiolabeled p-mGOT is present exclusively in the cytosol, and radiolabeled mature mGOT exclusively in the mitochondria.

When rat liver explants were pulse-labeled for 20 min and 50 min with [^{35}S]-methionine and subsequently chased for 30 min in the presence of unlabelled methionine, the amount of pulse-labeled mGOT in the mitochondrial fraction (mature form) increased during

chase (Fig 3B, lanes 3 and 5). In pulse and chase experiments, the amount of labeled p-mGOT in the cytosolic fraction was similar to, or less than that before chase (Fig 3A, lanes 3 and 5). On the contrary, the amount of labeled mature form in the ctyosolic fraction increased during chase (Fig 3A, lanes 3 and 5) in parallel with increase of labeled mature form in the mitochondrial fraction (Fig 3B, lanes 3 and 5). In other words, pulse-labeled p-mGOT in the cytosol decreased during chase, while mature mGOT in the mitochondria increased during chase. These results suggest that mGOT, as like other mitochondrial proteins in rat liver cells (9,11,12), is initially synthesized in the cytosol as a larger precursor form and is then translocated into mitochondria in association with post-translational proteolytic processing.

After chase for 30 min, pulse-labeled p-mGOT had not disappeared completely from the cytosol. This result is inconsistent with the report that the precursors of ornithine carbamyltransferase (9) and carbamylphosphate synthetase (11,12), disappeared during chase for 30 min. This suggests that p-mGOT has a longer half life for translocation into mitochondria than these other enzymes. However, its half life is probably not very long, because p-mGOT is not detectable in normal rat liver. These findings should be confirmed by studies using another system in which the penetration rate of radiolabeled amino acids is faster, such as, for example, a liver cell suspension or a cell culture system.

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