Vol. 124, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1984 Pages 44-50

MOLECULAR CLONING OF cDNA FOR RAT GLYCINE METHYLTRANSFERASE

Hirofumi Ogawa, Tomoharu Gomi, Toshihiro Horii*, Hideyuki Ogawa*, and Motoji Fujioka

Department of Biochemistry, Toyama Medical and Pharmaceutical University, Faculty of Medicine, Sugitani, Toyama 930-01, Japan

*Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

Received July 12, 1984

Summary: Using a highly purified preparation of glycine methyltransferase mRNA, double-stranded cDNA was synthesized and inserted into the PstI site of pBR322. The resulting recombinant DNA was used to transform E. coli χ1776 by conventional methods. Among tetracycline-resistant transformants, a number of colonies were found to contain cDNA sequence for glycine methyltransferase as examined by hybrid-selected translation. A restriction endonuclease cleavage map was constructed covering about 720 base pairs. With the cDNA as the probe, the content of the glycine methyltransferase mRNA was quantitated in various rat tissues and was found to be proportional to the specific enzyme activity.

Rat liver glycine methyltransferase (EC 2.1.1.20), which is composed of four identical subunits with M_r=31,500 (1), is an inducible enzyme (2). Feeding the rat with a high methionine-diet results in an increase in the amount of the enzyme in the liver along with an augmentation of activities of S-adenosylmethionine synthetase and S-adenosylhomocysteinase, which are involved in the transsulfuration pathway of methionine metabolism. In contrast, kidney glycine methyltransferase, which is immunologically identical with the liver enzyme is unaffected by this treatment (Hirofumi Ogawa, unpublished observation). It is also shown that the methyltransferase activity is virtually absent or very low in rat hepatoma cells and in the fetal and regenerating rat liver (3, 4). To understand the molecular mechanism of regulation of glycine methyltransferase and to assess its role in the sulfur metabolism, it seems useful to obtain cDNA for the enzyme. In this communication, we describe the isolation of clones containing glycine methyl-

0006-291X/84 $1.50

transferase cDNA sequence and the quantitation of the glycine methyltransferase mRNA in various rat tissues using the cDNA as the hybridization probe.

EXPERIMENTAL PROCEDURES

Materials. Biochemicals were obtained from the sources cited: avian myeloblastosis virus reverse transcriptase (Life Science and Mid-West Bio-Product), E. coli DNA polymerase I Klenow fragment (Biotec), terminal deoxynucleotidyl transferase (Takara Shuzo), S1 nuclease (Boehringer), nuclease Bal31 (Bethesda Research Laboratories), restriction endonucleases (Takara Shuzo, Nippon Gene and Toyobo), E. coli rRNA (Miles), nitrocellulose filters (Schleicher and Schüll), [α-^{32}P]dCTP, [^{3}H]dCTP and [^{35}S]methionine (Amersham). Other chemicals were of the highest purity available from local sources. Male Wistar strain rats (200 g) were purchased from Hokuriku Laboware (Toyama).

Purification of Glycine Methyltransferase mRNA. Messenger RNA coding for glycine methyltransferase was purified from free polysomes by specific immunoadsorption as described previously (5).

Synthesis, Selection, and Characterization of cDNA. Prior to the use for cDNA synthesis, the messenger RNA for glycine methyltransferase was thoroughly denatured. The purified mRNA (1 μg) was mixed with E. coli rRNA (20 μg) and treated with guanidium thiocyanate (6). The RNA recovered by centrifugation in an International 410 rotor for 24 h at 150,000 X g was further treated with 10 mM methylmercury hydroxide (7). Double-stranded cDNA was synthesized by the method of Wickens et al. (8) except that DNA polymerase I Klenow fragment was used in place of the intact polymerase. cDNA was made radioactive by using [^{3}H]dCTP. The double-stranded cDNA was trimmed with S1 nuclease, tailed with dCTP and terminal transferase (9), and inserted into the PstI site of pBR322 which had previously been dG-tailed. The recombinant DNA was used to transform E. coli χ1776 as described (9). The clones containing glycine methyltransferase cDNA sequence were identified by hybrid-selected translation (9).

For restriction endonuclease cleavage mapping, 0.5 μg of the cDNA insert was digested with one or two of various restriction endonucleases, and the resulting DNA fragments were analyzed by electrophoresis on 5% polyacrylamide gel with pBR322-HinfI digest as the size markers.

Dot-blot hybridization analysis was performed as described (10). Phenol/chloroform extracts (11) from various tissues except pancreas were used as RNA samples. Extraction of RNA from pancreas was made with guanidium thiocyanate.

Enzyme Assay. The glycine methyltransferase activity was determined as described previously (2). The supernatant obtained by centrifugation (10,000 X g, 15 min) of a tissue homogenate prepared in 50 mM potassium phosphate, pH 7.2, was used as the enzyme source.

Other Determinations. Protein was determined by the micro-biuret method (12) with bovine serum albumin as the standard. RNA was estimated by assuming an absorbance of 0.022 at 260 nm for 1 μg/ml solution. Double immunodiffusion on agarose gel was carried out by the method of Ouchterlony (13).

RESULTS

Cloning of Recombinant DNA for Glycine Methyltransferase. At the biginning of this work, we constructed a cDNA library using poly(A)-containing RNA which had been obtained from the phenol extract of whole liver by

oligo(dT)-cellulose chromatography and sucrose-density gradient centrifugation. The density gradient centrifugation resulted in about 5-fold enrichment over the total poly(A)-containing RNA with respect to the glycine methyltransferase mRNA. The cDNAs were inserted into pBR322 and used to transform E. coli. The transformants were screened by differential colony hybridization (14) using ^{32}P-labeled cDNAs to the liver and lung poly(A)-containing RNAs. Since glycine methyltransferase activity was not detected in the lung, it was assumed that this organ contained no mRNA for the enzyme. About 200 out of 800 clones were found to be liver-specific. Each of the liver-specific colonies were cultured independently and examined for the presence of the sequence complementary to the glycine methyltransferase mRNA by hybrid-selected translation. Only one colony, designated as pGMT 767, was found to have the cDNA sequence. Size estimation of the cDNA insert showed that it contained about 220 base pairs (data not shown), a value much smaller than that expected for the glycine methyltransferase mRNA (5). Since it is possible that the dG·dC-tails hinder the hybridization, the cDNA insert was digested from both sides with nuclease Bal31 to an average length of 120 base pairs, labeled by nick translation with ^{32}P-dCTP (9), and used as the hybridization probe for the selection of more clones from the library. However, about 50 clones selected by this probe were also found to contain the cDNA insert of the same length. The reason for this is not clear, but the possibility may be considered that the glycine methyltransferase mRNA contains a region which is refractory to the action of reverse transcriptase due to base pairing or by other reasons and, in the presence of many kinds of mRNAs, glycine methyltransferase cDNAs longer than 220 bases are difficult to obtain. Therefore, we decided to use a highly purified, thoroughly denatured preparation of glycine methyltransferase mRNA.

A highly purified preparation of glycine methyltransferase mRNA obtained by the immunoadsorption method (5) was treated with guanidium thiocyanate and methylmercury hydroxide and used to synthesize cDNA. The recombinant plasmid prepared by insertion of the cDNA into pBR322 was transfected to E. coli

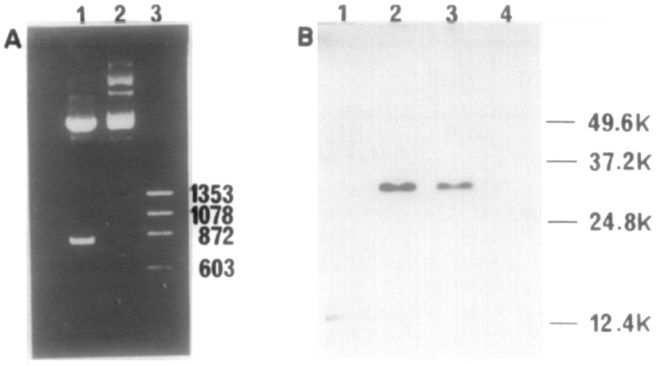

Fig. 1. A, agarose gel electrophoresis of the recombinant DNA and its PstI digest. The recombinant DNA (1 μg) before (lane 2) and after digestion with PstI (5 U) for 1 h at 37 °C (lane 1) was electrophoresed on 1.3% agarose gel. Lane 3 shows the electropherogram of HaeIII digest of ϕX174 DNA. B, identification of glycine methyltransferase cDNA by hybrid-selected translation. Plasmid DNA (10 μg) from pGMT A56 clone or from a negative clone was heat-denatured and spotted on a nitrocellulose filter (4 X 4 mm). The DNA was immobilized by baking and hybridized with 30 μg of poly(A)-containing RNA. The RNA arrested was eluted by boiling the filter and used for translation in a rabbit reticulocyte lysate. The translation products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography after immunoprecipitation with anti-glycine methyltransferase antiserum as described previously (5). Lane 3, with hybrid-selected RNA by pGMT A56; lane 4, with hybrid-selected RNA by the negative clone; lane 1, with no added RNA; lane 2, with total poly(A)-containing RNA. Cross-linked cytochrome c was used as the size marker.

X1776 as described under "Experimental Procedures". About 450 tetracycline-resistant colonies were picked up, grown separately on nitrocellulose filters, and screened for the presence of glycine methyltransferase cDNA sequence by colony hybridization. Single-stranded ^{32}P-labeled cDNA obtained from pGMT 767 as above was used as the hybridization probe. Thirty-six clones gave an intense signal. One of the clones (pGMT A56) was found to contain cDNA having more than 700 base pairs (Fig. 1A). To confirm that the clone has the glycine methyltransferase cDNA sequence, hybrid-selected translation was carried out. The selected mRNA directed the synthesis of a polypeptide which was immunoprecipitable with anti-glycine methyltransferase antibody and had an identical mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the authentic glycine methyltransferase subunit (Fig. 1B).

Restriction Endonuclease Cleavage Map of Glycine Methyltransferase cDNA Insert. The restriction endonuclease cleavage map of the cDNA insert in pGMT A56 is shown in Fig. 2. The insert had 5 and 7 cleavage sites for HpaII and

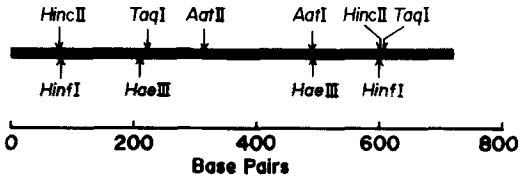

Fig. 2. Restriction map of the glycine methyltransferase cDNA insert of pGMT A56.

BstN1, respectively, but was not cleaved by AluI, BamH1, BglII, EcoRI, HindIII, PvuIII, XbaI, and XhoI. The insert was found to have 720 base pairs.

Quantitation of Glycine Methyltransferase mRNA in Various Tissues. Kerr reported that a significant activity of glycine methyltransferase was found only in the liver, kidney, and pancreas in the rat (3). We confirmed her observation. The same enzyme protein appears to be responsible for the activities in these tissues as evidenced by the double immunodiffusion test (Fig. 3A). The brain showed no enzyme activity nor had cross-reacting material. When the amount of glycine methyltransferase mRNA in various tissues was determined by dot-blot hybridization, a close correlation was found between the enzyme specific activity and the mRNA content (Fig. 3B). Those tissues

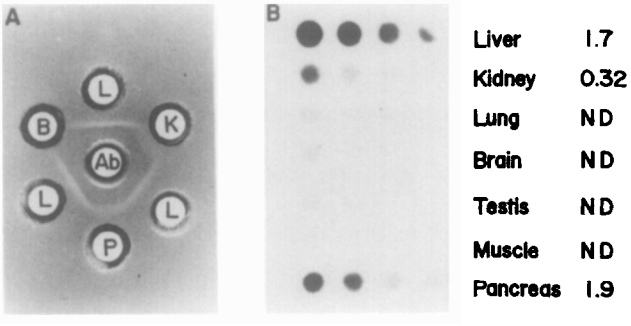

Fig. 3. A, double immunodiffusion on agarose gel. The center well (Ab) contained anti-glycine methyltransferase antiserum raised in rabbits against the purified liver enzyme. The outer wells contained extracts of the liver (L), pancreas (P), kidney (K), and brain (B). B, dot-blot hybridization analysis. Poly(A)-containing RNAs extracted from various tissues were spotted (2, 1, 0.5, and 0.25 µg; from left to right) on a nitrocellulose filter and immobilized. The filter was incubated in the hybridization buffer (10) containing ^{32}P-labeled cDNA (obtained from pGMT 767) at 42 °C overnight. After washing with sodium citrate/sodium chloride (10), the filter was subjected to autoradiography. The numbers on the right show the enzyme activities expressed as units/min/mg protein (1). ND, activity not detected.

showing no glycine methyltransferase activity (lung, brain, testis, and muscle) had no mRNA for the enzyme.

DISCUSSION

We have isolated clones containing cDNA sequence for glycine methyltransferase. This is, as far as we know, the first example of cloning of a mammalian S-adenosylmethionine-dependent methyltransferase cDNA.

Initially, we obtained by hybrid-selected translation a clone (pGMT 767) having the glycine methyltransferase cDNA sequence from a cDNA library constructed with the liver poly(A)-containing RNA. The cDNA insert contained in the clone, however, had only 220 base pairs. It is reported that the hybrid-selected translation procedure sometimes selects small cDNAs preferentially because the mRNA hybridized through a small region would be released more readily from the cDNA (15). Our failure to obtain larger cDNA inserts appears not due to this phenomenon. The clone identified by colony hybridization with the pGMT 767 cDNA as the probe invariably contained 220 base pairs. Since this suggested a difficulty in transcribing the glycine methyltransferase mRNA by the reverse transcriptase, we started with a highly purified, denatured preparation of mRNA for the synthesis of cDNA and colonies containing the recombinant DNA were identified by colony hybridization. A number of clones having larger cDNA inserts were obtained by this procedure. The largest cDNA insert contained 720 base pairs covering 52% of the total length of the mRNA (5). The cDNA may be considered to contain about 43% of the coding region.

The dot-blot hybridization experiment showed that the activity of glycine methyltransferase in a rat tissue was proportional to the mRNA content. Whether the induction of the enzyme by high methionine-diet is due to the increase of mRNA or to the augmentation of translational efficiency is now under investigation.

ACKNOWLEDGMENTS

We express our thanks to Dr. N. Itoh for valuable advices at an early stage of this work. This investigation was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Cultue of Japan.

REFERENCES

1. Ogawa, H., and Fujioka, M. (1982) J. Biol. Chem. 257, 3447-3452.
2. Ogawa, H., and Fujioka, M. (1982) Biochem. Biophys. Res. Commun. 108, 227-232.
3. Kerr, S. J. (1972) J. Biol. Chem. 247, 4248-4252.
4. Liau, M. C., Chang, C. F., Geianger, L., and Grenier, A. (1977) Cancer Res. 39, 162-169.
5. Gomi, T., Ogawa, H., and Fujioka, M. (1984) Biochem. Internatl. 9, 25-31.
6. Chirgwin, J. M., Przybyla, A. E., MacDonald, R., and Rutter, W. (1979) Biochemistry 18, 5294-5299.
7. Bailey, J. M., and Davidson, N. (1976) Anal. Biochem. 70, 75-85.
8. Wickens, M. P., Buell, G. N., and Schimke, R. T. (1978) J. Biol. Chem. 253, 2483-2495.
9. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor.
10. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5201-5205.
11. Itoh, N., Nose, K., and Okamoto, H. (1979) Eur. J. Biochem. 97, 1-9.
12. Itzhaki, R. F., and Gill, D. M. (1964) Anal. Biochem. 9, 401-410.
13. Ouchterlony, O. (1949) Acta Pathol. Microbiol. Scand. 26, 507-515.
14. Grunstein, M., and Hogness, D. S. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3961-3965.
15. Matteson, K. J., Chung, B., and Miller, W. L. (1984) Biochem. Biophys. Res. Commun. 120, 264-270.