Purification and Properties of Glycine N-Methyltransferase from Rat Liver*

Hirofumi Ogawa and Motoji Fujioka
From the Department of Biochemistry, Toyama Medical and Pharmaceutical University, Faculty of Medicine, Sugitani, Toyama 930-01, Japan

Glycine N-methyltransferase (EC 2.1.1.20) has been purified to homogeneity from rat liver. The enzyme has a molecular weight of 132,000 by sedimentation equilibrium method. This value is in good agreement with a value of 130,000 obtained by Sephadex G-150 chromatography. The molecular weight of the denatured enzyme as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate is 31,500. The numbers of peptides obtained by trypic digestion and by cyano gen bromide cleavage are four-identity of those expected from the contents of lysine plus arginine residues and methionine residues, respectively. By Edman degradation, phenylthiodyantoin-leucine is the only amino acid derivative released from the enzyme. Neither sugar nor phospholipid is detected in the purified preparation. These data indicate that the rat liver glycine N-methyltransferase is a simple protein consisting of four identical subunits. The enzyme has an isoelectric pl of 6.4, and is most active at pH 9.0. From the circular dichroism spectrum, an a helix content of about 11% is calculated. Whereas the initial velocity as a function of glycine concentration gives a Michaelis-Menten kinetics, the enzyme shows a positive cooperativity with respect to S-adenosylmethionine. The concentrations of glycine and S-adenosylmethionine which give a half-maximum velocity are 0.13 mM and 30 p~m, respectively, at pH 7.4 and 25 °C.

Biological transmethylation reactions utilizing S-adenosylmethionine as a methyl donor have attracted the attention of many biochemists since the recognition that they are involved in a variety of cellular processes through methylation of nucleic acids, proteins, phospholipids, and some small molecules (see, for example, Ref. 1). Although a number of methyltransferases have been purified and their functions examined, the physiological function of glycine N-methyltransferase (EC 2.1.1.20) which catalyzes the S-adenosylmethionine-dependent methylation of glycine to yield sarcosine is not well understood.

Glycine N-methyltransferase was first found in an extract of guinea pig liver in 1960 by Blumenstein and Williams (2). They postulated that the enzyme is involved in the oxidation of methyl carbon of methionine since sarcosine can be oxidatively cleaved to one-carbon unit and glycine by sarcosine dehydrogenase. Later studies, however, have shown that the metabolism of methionine methyl carbon by this pathway accounts for only 20% of the total (3), thus questioning the role of glycine N-methyltransferase in the methionine metabolism. On the other hand, Kerr (4), from her studies on tRNA methylation, has suggested that the enzyme may play a role in the regulation of relative levels of S-adenosylmethionine and S-adenosylhomocysteine in the cell.

Headly and Kerr (5) have purified glycine N-methyltransferase from rabbit liver to apparent homogeneity, and have shown that it is a glycoprotein consisting of 3 to 4 nonidentical subunits with molecular weights in the range of 27,000 to 33,000. They have also reported that the enzyme represents as much as 0.9 to 3% of the soluble protein of rabbit liver.

In order to understand more fully the molecular architecture of the enzyme and to gain an insight into its role in the cellular metabolism, we have decided to purify and to characterize the enzyme from rat liver. In the present paper, we describe the purification procedure for glycine N-methyltransferase from rat liver and report on its molecular and catalytic properties which turned out to be considerably different from those of the rabbit liver enzyme.

EXPERIMENTAL PROCEDURES AND RESULTS*

Criteria for Purity

Polyacrylamide Gel Electrophoresis—The purified enzyme preparation showed a single protein band on SDS-polyacrylamide gel electrophoresis (Fig. 1). When electrophoresed (7.5% polyacrylamide gel; 20 μg of protein) in the absence of the detergent (6), it also showed a single protein band with an RF of 0.17 (not shown).

Ultracentrifugal Analysis—The purified enzyme showed single sedimenting boundaries in ultracentrifuge (not shown). The sedimentation coefficient at 20 °C and a protein concentration of 0.3 mg/ml was 5.8. In sedimentation equilibrium runs, the plot of ln concentration versus r² gave a straight line (Fig. 2).

Immunological Purity—Double immunodiffusion tests performed on agarose gel with the antiserum prepared in rabbit by immunizing the purified enzyme are shown in Fig. 3. As the figure shows, the antiserum formed a single precipitin line with a crude liver extract as well as with the purified enzyme, indicating the immunological homogeneity of the purified enzyme.

* Portions of the paper (including "Experimental Procedures," "Results," Tables I and II, Figs. 2, 7, and 9, and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 81M-1894, cite the authors and include a check for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

† The abbreviation used is: SDS, sodium dodecyl sulfate.

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FIG. 1. SDS-polyacrylamide gel electropherograms of samples at each stage of purification. Electrophoresis was carried out on 10% polyacrylamide gel by the method of Laemmli. 1, acid supernatant; 2, ammonium sulfate; 3, DEAE-cellulose; 4, CM-Sephadex; 5, hydroxylapatite (10 μg of protein).

FIG. 3. Double immunodiffusion test on agarose gel. Well 1 contained the antiserum to glycine N-methyltransferase, and Wells 2 and 3 contained a crude rat liver extract and the purified enzyme, respectively. An unimmunized serum was placed in Well 4.

The data presented above and the fact that only one phenylthiohydantoin-amino acid is detected in the Edman degradation (see below) together establish the homogeneity of the purified enzyme.

Molecular Properties

Molecular Weight—The molecular weight of glycine N-methyltransferase was estimated by Sephadex G-150 gel filtration with several standard proteins (7) and by sedimentation equilibrium method (8). In gel filtration chromatography, the enzyme was eluted from the column as a single symmetrical peak with a $K_v$ value of 0.27, corresponding to a molecular weight of 130,000 (Fig. 4A). By sedimentation equilibrium method a molecular weight of 132,000 (an average of 2 runs) was obtained, assuming a partial specific volume of 0.751 ml/g calculated from the amino acid composition (9).

The molecular weight of the denatured enzyme was determined by SDS-polyacrylamide gel electrophoresis as described by Weber and Osborn (10), and a value of 31,500 (an average of 2 determinations) was found (Fig. 4B). These data suggest that glycine N-methyltransferase is composed of 4 subunits.

Amino Acid Composition—The results of amino acid analysis of the enzyme is presented in Table II. All of the common amino acids were present. No amino sugar was detected even with a 16-h hydrolysate. Half-cystines appear to be exclusively cysteines, since 16 sulfhydril groups/mol of enzyme were titratable with 5,5'-dithiobis(2-nitrobenzoate) (11) under denaturing conditions. In the absence of SDS, a total of 12 sulfhydril groups/mol of enzyme were found, suggesting that 4 cysteines are buried and not accessible to the reagent.

NH$_2$-terminal and COOH-terminal Amino Acids—The NH$_2$-terminal amino acid of glycine N-methyltransferase was found to be leucine by the Edman degradation procedure (12). The enzyme preparation which had been dialyzed against water and lyophilized was practically insoluble in dimethylalylamine buffer, but the addition of 2-mercaptoethanol to a final concentration of 1% was found to solubilize the sample. Identification of the penultimate residue was not possible because the peptide remaining after the first cycle resisted all our efforts to solubilize it.

The COOH-terminal residue was determined by digestion with carboxypeptidase A. As Fig. 5 shows, the peptidase released glycine, followed by threonine and lysine. Small amounts of leucine and valine were also found. The amount of glycine released after a 2-h incubation was about 4 mol/mol of enzyme, in accordance with a tetrameric structure proposed by the molecular weight determinations. The results of end group analyses strongly suggest that glycine N-methyltransferase contains 4 identical subunits whose COOH-terminal sequence is -Lys-Thr-Gly.

Peptide Analysis—To further establish the identity of the subunits, peptide analyses were carried out with the tryptic peptides and with the cyanogen bromide peptides. Glycine N-methyltransferase contains 68 lysine and 60 arginine residues/mol of enzyme (Table II). Therefore, if the 4...
subunits of the enzyme are identical, the tryptic peptide map is expected to show 33 peptide spots. As shown in Fig. 6, the number of spots obtained (about 30) is very close to the expected value.

Likewise, from the methionine content, cyanogen bromide should cleave the enzyme into 5 peptides and these would be seen on polyacrylamide gel electrophoresis if methionine residues do not occur at distal positions of the polypeptide chain and are not clustered. Examination of the cleavage product on SDS-polyacrylamide gel electrophoresis as described under “Experimental Procedures” revealed the presence of 5 peptides with molecular weights ranging from 4,000 to 14,000. Furthermore, the sum of the molecular weights of these peptides was in reasonable agreement with the subunit molecular weight (Mr = 31,500).

Other Properties—The isoelectric pH of the enzyme was determined to be 6.4 by isoelectric focusing.

Glycine N-methyltransferase had an absorption maximum at 278 nm; no absorption was observed in the visible region (Fig. 7A). The CD spectrum of the enzyme revealed a negative CD in the ultraviolet region with a minimum at 221 nm (Fig. 7B). From the mean residue ellipticity at 222 nm, the a-helix content was calculated to be approximately 11% (13).

Heady and Kerr (5) reported that the glycine N-methyltransferase from rabbit liver was a glycoprotein. The possibility that the rat liver enzyme is also a glycoprotein was examined by staining the polyacrylamide gel after electrophoresis with periodic acid-Schiff reagent (14). No periodic acid-Schiff positive material was observed, however. Ovalbumin treated similarly showed the presence of sugar, as expected. Phospholipid was also not detected when analyzed with 300 μg of the enzyme protein.

Catalytic Properties

Substrate Specificity—The glycine N-methyltransferase from rat liver was highly specific for glycine as the methyl acceptor. None of the common L-amino acids were methylated when tested at a concentration of 5 mM L-Homoserine, L-ornithine, glycylglycine, glycynamide, glycine ethylester, and ethanalamine were also inert as substrates.

Kinetic Properties—The effect of substrate concentration on reaction rate was examined at pH 7.4 and 25 °C. With S-adenosylmethionine as the variable substrate at a constant concentration of glycine (1.0 mM), the plot of initial velocities against substrate concentrations exhibited a sigmoidal curve (Fig. 8A). From a Hill plot (15) of the same data, a maximum Hill coefficient of 2.2 was obtained. In order to test a possible dependence of kinetics on the concentration of glycine, experiments were performed at several fixed concentrations of glycine ranging from 0.05 to 3.0 mM. At all glycine concentrations tested, however, positive cooperativity was observed with a nearly identical Hill coefficient (data not shown).

In contrast to S-adenosylmethionine, glycine showed hyperbolic kinetics at all fixed concentrations of S-adenosylmethionine (Fig. 8B). The hyperbolic nature of the kinetics was confirmed by carrying out the experiments over a very wide range of glycine concentration at a constant level of S-adenosylmethionine (0.1 mM) and calculating the ratio of glycine concentrations giving 90 and 10% of the maximum activity (16). No deviation from hyperbolic kinetics was observed.

The apparent values of S0.5 (the concentration which gives a half-maximal activity) for S-adenosylmethionine and K, for glycine were dependent on the concentration of the other substrate. The values decreased with increasing concentrations of the second substrate. The limiting values obtained at saturating concentration of the other substrate were 30 μM.

![Fig. 8.](http://www.jbc.org/)
and 0.13 mM for S-adenosylmethionine and glycine, respectively.

The positive cooperativity with respect to S-adenosylmethionine was also noted at pH 8.8. S-Adenosylhomocysteine is known to be a potent inhibitor of a variety of transmethylation reactions. The rat liver glycine N-methyltransferase was found to be very susceptible to inhibition by this compound; a 50% inhibition was obtained at a concentration of about 20 \( \mu \)M, respectively. The enzyme was not inhibited by 2 mM, respectively. The enzyme was not inhibited by any of a variety of transmethylation reactions. The rat liver glycine N-methyltransferase was purified approximately 1,300-fold from the rat liver extract with a recovery of about 38%. The purified enzyme appears to be homogeneous from several accepted criteria for purity.

The molecular weights of the native and denatured enzymes show that the enzyme is an oligomeric protein consisting of 4 subunits. The presence of single NH\(_2\)- and COOH-terminal amino acid residues and the fact that the numbers of peptides obtained by tryptic digestion and cyanogen bromide cleavage are one-fourth of those expected from the contents of lysine plus arginine residues and methionine residues, respectively, indicate that the 4 subunits of the enzyme are identical. This result is in sharp contrast to the molecular structure of the rabbit liver enzyme. Heady and Kerr (5) reported that the rabbit liver enzyme had a similar molecular weight of 123,500, but consisted of 3 to 4 nonidentical subunits. Another difference in molecular properties between the enzymes from the two sources is the absence of sugar in the rat liver enzyme. No carbohydrate was detected with periodic acid-Schiff stain or in amino acid analysis.

Unlike the rabbit liver enzyme which shows Michaelis-Menten kinetics for both substrates (5), the glycine N-methyltransferase from rat liver exhibits a positive cooperativity with respect to S-adenosylmethionine (Fig. 8). Since the cooperativity is observed only with S-adenosylmethionine as the variable substrate, possibilities may be considered that the observed kinetics is due to the instability of S-adenosylmethionine at low concentrations and to the failure of phosphotungstic acid to trap all of the S-adenosylmethionine remaining when its concentrations are high (see "Experimental Procedures"). The first possibility is unlikely because a precipitation of S-adenosylmethionine in the absence of enzyme has no effect on the reaction rate. The second possibility could be ruled out by use of a different assay method. The glycine methyltransferase reaction was carried out in the presence of high concentrations of S-adenosylhomocysteine (EC 3.3.1.1) and adenosine deaminase (EC 3.5.4.4) and the change in absorbance at 265 nm resulting from the conversion of adenosine to inosine was monitored. Cooperativity was also obtained by this spectrophotometric assay.

The \( K_m \) value for glycine currently obtained at a physiological pH is well below the tissue level of the compound (17), and hence, the enzyme may be considered to be saturated with glycine in vivo. On the contrary, the concentration range of S-adenosylmethionine which shows maximum reaction rate in the rat liver is comparable to the tissue content of S-adenosylmethionine (18). From these observations, it may be suggested that one possible function of the enzyme is to regulate the cellular levels of S-adenosylmethionine and/or S-adenosylhomocysteine, and eventually transmethylation reactions.

Although glycine N-methyltransferase appears to make a limited contribution in the metabolism of methionine (3), sarcosine formed by the action of the enzyme may be of some importance in one-carbon metabolism, or in the synthesis of glycine and serine in mitochondria. Wittwer and Wagner (19) have recently reported on an abundant occurrence of sarcosine dehydrogenase in rat liver mitochondria, which catalyzes the cleavage of sarcosine to glycine and 5,10-methylenetetrahydrofolate in the presence of tetrahydrofolate. The 5,10-methylenetetrahydrofolate thus formed would enter the mitochondrial pool of one-carbon unit, and the glycine would be utilized for synthesis of serine (20) and heme (21), or be degraded to \( \text{CO}_2 \) and \( \text{NH}_3 \) by the glycine cleavage system present in mitochondria (22). Further studies are needed in understanding the physiological role of glycine N-methyltransferase.

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REFERENCES
1. Usdin, R., Borchardt, R. T., and Creweling, C. R., eds (1979) Transmethylation, Elsevier North Holland, New York
2. Blumenstein, J., and Williams, G. R. (1960) Biochem. Biophys. Res. Comm. 3, 259–263
3. Mitchell, A. D., and Benevenga, N. J. (1976) J. Nutr. 106, 1721–1726
4. Kerr, S. J. (1972) J. Biol. Chem. 247, 4248–4252
5. Heady, J. E., and Kerr, S. J. (1973) J. Biol. Chem. 248, 69–72
6. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 122, 404–427
7. Andrews, P. (1965) Biochem. J. 96, 595–606
8. van Holde, K. E., and Baldwin, R. L. (1958) J. Phys. Chem. 62, 734–743
9. Schachman, H. K. (1957) Methods Enzymol. 4, 32–103
10. Weber, K., and Osborn, J. (1966) J. Biol. Chem. 244, 4406–4412
11. Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70–77
12. Edman, P. (1958) Acta Chem. Scand. 4, 277–282
13. Chen, Y.-H., Yang, J.-Y., and Martinez, H. M. (1972) Biochemistry 11, 4120–4134
14. Zacharias, R. M., Zell, T. E., Morrison, J. H., and Woodlock, J. J. (1969) Anal. Biochem. 30, 148–152
15. Hill, A. J. (1913) Biochem. J. 7, 471–480
16. Kosher, D. E., Jr., Nemethy, G., and Filmer, D. (1966) Biochemistry 5, 365–385

Table III
Summary of physicochemical and enzymological properties of glycine N-methyltransferase

| Property                              | Value       |
|---------------------------------------|-------------|
| Molecular weight                      | 132,000     |
| Sedimentation equilibrium             | 130,000     |
| Gel filtration                        | 31,500      |
| Gel electrophoresis*                  |             |
| Partial specific volume               | 0.751 ml/g  |
| NH\(_2\) terminus                      | leucine     |
| COOH terminus                         | glycine     |
| \([\theta]_280\)                       | −6510 deg cm\(^2\) dmol\(^−1\) |
| \(\alpha\) Helix content              | 11%         |
| Isoelectric pH                        | 6.4         |
| Optimum pH                            | 9.0         |
| Kinetic constants                     |             |
| \(V_{max}\)                           | 174 nmol min\(^−1\) ng\(^−1\)|
| \(S_0.5\) (S-adenosylmethionine)      | 30 \(\mu\)M |
| \(K_m\) (glycine)                     | 0.13 \(\mu\)M |

* SDS-polyacrylamide gel electrophoresis.
‡ From amino acid composition.
§ Calculated from the equation of Chen et al. (13).
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**EXPERIMENTAL PROCEDURES**

**Materials**
- The enzyme reactions and other materials were purchased from the sources cited: hydroxyapatite (Bio-Rad, Richmond, CA); Sephadex G-75 (Pharmacia, Uppsala, Sweden); and Sephadex G-200 (Pharmacia, Uppsala, Sweden) 
- Sodium diethylthiocarbamate (2,5-dithiopropionic acid) (Eastman Organic Chemicals, Rochester, NY) 
- Triton X-100 (Triton-X-100) (Sigma Chemical Co., St. Louis, MO) 
- Cellulose phosphate ester (Cellulose phosphate ester, 30000 MW) (Serva, Heidelberg, West Germany) 

**Preparation of Enzyme**
The enzyme reaction mixture was preincubated at 0°C for 5-10 minutes before assay.

**Assay of Enzyme Activity**
- The reaction mixture contained the following components in a final volume of 0.5 ml: 
  - 20 mM sodium phosphate buffer, pH 7.5 
  - 0.2 mM glycine 
  - 0.07 mM NADPH 
  - 0.02 mM L-serine 
  - 0.02 mM 5,6-dichloro-2-phenyltetrazolium bromide

**Enzyme Purification**
- The purification procedure was as follows: 
  - Step 1: DEAE-cellulose chromatography 
  - Step 2: Phosphocellulose chromatography 
  - Step 3: Calcium phosphate column chromatography 
  - Final purification

**Results**

**TABLE I**

| Step | Protein | NADPH consumed | Activity |
|------|---------|----------------|----------|
| Original | 1,430 | 63.3 | 0.12 |
| DEAE-cellulose | 195 | 53.0 | 0.17 |
| Phosphocellulose | 65 | 21.8 | 0.04 |
| Calcium phosphate column | 35 | 15.0 | 0.06 |
| Final purification | 30 | 0.4 | 160.3 |

**Discussion**
- The enzyme activity was measured by the formation of glycine N-methyltransferase.
- The purified enzyme was used in the experiments with a specific activity of 160.3.

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**References**

1. Bloxam, D. L., and Wagner, A. S. (1977) J. Biol. Chem. 252, 8683-8688
2. Wittwer, A. J., and Wagner, C. (1981) J. Biol. Chem. 256, 4209-4215
3. Ogawa, H., and Fujioka, M. (1981) J. Biochem. (Tokyo) 90, 381-385
4. Eloranta, T. O., and Raina, A. M. (1977) Biochem. J. 168, 179-185
5. Bloxam, D. L., and Wagner, A. S. (1981) Br. J. Nutr. 27, 233-247
6. Rose, R. E., and Young, J. E. (1973) J. Biol. Chem. 248, 4797-4802
7. Wittwer, A. J., and Wagner, C. (1981) J. Biol. Chem. 256, 4209-4215
8. Eloranta, T. O., and Raina, A. M. (1977) Biochem. J. 168, 179-195
9. Wittwer, A. J., and Wagner, C. (1981) J. Biol. Chem. 256, 4209-4215
10. Ogawa, H., and Fujioka, M. (1981) J. Biochem. (Tokyo) 90, 381-385
Fig. 1. Sedimentation equilibrium of glycine N-methyltransferase. The logarithm of concentration ($c$) is plotted against the square of distance ($r^2$) from the center of rotation ($g$).

Fig. 2. Effect of pH on reaction rate. The activity measurements were made at 25°C in 0.1 M Tris-HCl at pH values indicated.

Fig. 3. Absorption and CD spectra of glycine N-methyltransferase. A, absorption spectrum. The spectrum was recorded at a protein concentration of 0.1 mg/ml of 10 mM potassium phosphate, pH 7.2. B, CD spectrum. Conditions are described under "Experimental Procedure".
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