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Cooperative interactions of tetrahydrofolate with purified pig kidney serine transhydroxymethylase and loss of this cooperativity in L1210 tumors and in tissues of mice bearing these tumors

(enzyme kinetics/desensitization/5,10-methylenetetrahydrofolate)

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ABSTRACT **Serine transhydroxymethylase (5,10-methylenetetrahydrofolate: glycine hydroxymethyl transferase, EC 2.1.2.1) purified 200-fold from pig kidneys showed cooperative interactions with tetrahydrofolate with a Hill coefficient (*n* value) of 3.9 and a substrate concentration at 50% of maximum velocity, the $S_{0.5}$ value, of 0.5 mM. The enzyme in mouse liver and kidney homogenates also showed cooperative interactions with tetrahydrofolate. However, the enzyme obtained from L1210 solid tumors of mice, and from livers and kidneys of mice inoculated with L1210 cells exhibited hyperbolic saturation kinetics and gave a Michaelis constant, K_m, value of 0.5 mM for tetrahydrofolate. The interaction of serine with the enzyme from pig kidney, from tissues of normal or tumor-bearing mice, or from L1210 tumors was hyperbolic with a K_m of 0.9 mM. The specific activities of the enzyme in the L1210 tumor and in mouse liver were 10-fold higher than in pig or mouse kidney. There was no significant change in the levels of the enzyme in mouse liver and kidney on inoculation with L1210 cells.**

These results suggest that a tumor can bring about biochemical changes in tissues that are distal to the tumor.

Serine transhydroxymethylase (EC 2.1.2.1) catalyzes the transfer of the hydroxymethyl group from serine to 5,6,7,8-tetrahydrofolate to yield 5,10-methylenetetrahydrofolate and glycine as products of the reaction. 5,10-Methylenetetrahydrofolate is a common precursor for: the biosynthesis of the methyl group of methionine (1); thymidylate (2); purine carbon atoms 2 and 8 (3); the formyl group of formylmethionyl-tRNA (4); and the hydroxymethyl group of hydroxymethyl-dCMP and hydroxymethyl-dUMP (5, 6). Serine transhydroxymethylase isolated from a number of mammalian sources (7, 8) has a molecular weight of 215,000, is polymeric (9), and binds 4 moles of pyridoxal 5′-phosphate (pyridoxal-*P*) per mole of enzyme (10). This enzyme has been purified from bacteria (11, 12) and in *Escherichia coli* enzyme synthesis is repressed by methionine (13). Most often in the study of the mechanism of this enzyme from mammalian systems (14) glycine and formaldehyde or threonine are used as substrates. In this communication, we report the allosteric interactions of tetrahydrofolate with serine transhydroxymethylase purified from pig kidney, and in homogenates of mouse liver and kidney; and the absence of these cooperative effects in the enzyme obtained from L1210 solid tumors, or from livers and kidneys of mice containing these tumors.

MATERIALS AND METHODS

Pyridoxal 5-phosphate, ethylenediaminetetraacetate (EDTA), dithiothreitol, 2-mercaptoethanol, and 5,5-dimethyl-1,3-cyclohexanedione (dimedone) were obtained from Sigma Chemical Co; carboxymethyl- (CM)-Sephadex C-50, from Pharmacia Fine Chemicals Inc.; [3-^{14}C]serine from New England Nuclear Corp.; and pig kidneys, from the local slaughterhouse. Tetrahydrofolate was prepared by the catalytic hydrogenation of folic acid by the method of O'Dell *et al.*, as modified by Hatefi *et al.* (15).

L1210 solid tumors were induced by injecting an L1210 culture subcutaneously into the flanks of mice (strain BDF_1). After 7 days the solid tumors were dissected out and frozen immediately in liquid nitrogen. They were kept frozen at −70° until use.

Assay for Serine Transhydroxymethylase. The reaction mixture (0.55 ml) contained: 30 μmol of potassium phosphate buffer, pH 7.4; 0.1 μmol of pyridoxal-*P*; 1 μmol of tetrahydrofolate dissolved in 0.3 M potassium phosphate buffer at pH 7.4 which also contained 1 mM dithiothreitol; and an appropriate amount of the enzyme. The mixture was initially incubated for 5 min at 37° and then 0.2 μmol of L-[3-^{14}C]serine (3×10^6 cpm/μmol) was added and the incubation was continued for an additional 15 min at 37°. The reaction was stopped by adding 0.3 ml of dimedone (0.4 M in 50% ethanol) and by placing the tubes in a boiling-water bath for 5 min. The tubes were cooled for 5 min in ice, and the formaldehyde-dimedone adduct was extracted into 3 ml of toluene by vigorous shaking on a Vortex mixer for 30 sec. The tubes were then centrifuged for 2 min and 1.0 ml of the toluene layer was mixed with Aquasol (10 ml) and radioactivity was measured in a Tri-Carb liquid scintillation spectrometer. This procedure is essentially similar to that described by Taylor and Weissbach (16).

One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol of formaldehyde per hr at 37° and at pH 7.4.

Purification of Serine Transhydroxymethylase from Pig Kidney. Frozen pig kidneys (1.2 kg) were thawed, cut into cubes of 1 inch (2.5 cm) and homogenized with 3.5 liters of 0.03 M potassium phosphate buffer (pH 7.4) in a Waring Blendor for 1 min. To the crude extract (Table 1) obtained on centrifugation at 35,000 × *g* for 30 min, solid ammonium sulfate (300 g/2.2 liters) was added to achieve 0.25 saturation. The precipitate obtained on centrifugation at 35,000 × *g* was discarded. Solid ammonium sulfate (340 g/2.3 liters) was added to the supernatant solution to raise the saturation to 0.5. The precipitate was collected by centrifugation at 35,000 × *g* for 15 min, dissolved in 0.05 M phosphate buffer (pH 7.4) containing 10 μM pyridoxal-*P*, 20 mM EDTA, and 1 mM 2-mercaptoethanol, and dialyzed for 24 hr against 4 liters of the same buffer. The clear supernatant solution (ammonium sulfate fraction, Table 1) was layered on a CM-Sephadex C-50 column (3 × 32 cm) previously equilibrated with 0.05 M phosphate buffer (pH 7.4)

Abbreviations: pyridoxal-*P*, pyridoxal 5′-phosphate; CM-Sephadex, carboxymethyl-Sephadex; dimedone, 5,5-dimethyl-1,3-cyclohexanedione.

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Table 1. Purification of serine transhydroxymethylase from pig kidney

Fraction	Total activity, units*	Specific activity, units/mg of protein	Fold purification	Recovery, %
Crude extract	633	0.012	1	100
Ammonium sulfate precipitate	500	0.060	5	94
CM-Sephadex eluate	561	2.4	200	88

Purification of serine transhydroxymethylase from pig kidney. The following pig kidney preparations—the crude extract (3.8 mg of protein), the ammonium sulfate precipitate (3.4 mg of protein), or the CM-Sephadex eluate (200 μg of protein)—were preincubated for 5 min at 37° with tetrahydrofolate (1.5 mM). The reaction was started by the addition of 0.2 μmol of L-[3-^{14}C]serine and the enzyme was assayed by estimating the formaldehyde formed by the method of Taylor and Weissbach (16). Protein was determined by the biuret method (19).

* Micromoles of formaldehyde formed per hr.

containing pyridoxal-*P* (10 μM), EDTA (20 mM), and 2-mercaptoethanol (1 mM). The protein was eluted from the column with a linear gradient of 0.05–0.5 M phosphate buffer, pH 7.4. These buffers also contained pyridoxal-*P* (10 μM), EDTA (20 mM), and 2-mercaptoethanol (1 mM). The fractions containing serine transhydroxymethylase activity were pooled and used without any additional processing in these studies. The results of a representative purification procedure are summarized in Table 1. This procedure resulted in a 200-fold purification with an 88% recovery. This preparation was *not* contaminated with the following folate-requiring enzymes: methionine synthetase, 5,10-methylenetetrahydrofolate reductase, and 5,10-methylenetetrahydrofolate dehydrogenase.

Preparation of the Enzyme Extract from Mouse Kidney and Liver, and from L1210 Solid Tumors. The liver and kidneys were dissected from both normal and tumor-bearing mice and homogenized for 1 min in a Waring Blendor with 2 volumes of 0.3 M potassium phosphate buffer (pH 7.4). The supernatant solution obtained on centrifugation at 35,000 × *g* for 15 min was used as the enzyme source. In the case of the tumors, 50 g of frozen tumor tissue were allowed to thaw at room temperature and homogenized with 100 ml of 0.3 M potassium phosphate buffer, pH 7.4, for 1 min in a Waring Blendor. The supernatant solution obtained upon centrifugation at 35,000 × *g* provided the tumor serine transhydroxymethylase used in these studies.

RESULTS

Cooperative Interactions of Tetrahydrofolate with Pig Kidney Serine Transhydroxymethylase. The enzyme (200 μg) was preincubated for 5 min with tetrahydrofolate (0.04–1.0 μmol) and assayed for activity. From Fig. 1, which represents the relationship between the reciprocal of the substrate concentration and reciprocal of initial velocity, it is evident that cooperative effects are present. A sigmoid curve (not shown in Fig. 1) was obtained when initial velocity was plotted against tetrahydrofolate concentration. The *n* value calculated from the Hill plot (insert Fig. 1) was 3.9. The substrate concentration at 50% of maximum velocity, the $S_{0.5}$ value, was 0.5 mM.

The enzyme was treated with urea (6 M) and the urea was subsequently removed by extensive dialysis. When the renatured enzyme was assayed at the concentrations of tetrahydrofolate indicated in Fig. 1, a linear Lineweaver–Burk plot (not shown in the figure) was obtained. The *v* versus [S] plots were hyperbolic. The Hill plot gave a Hill coefficient (*n* value) of 1. The K_m for tetrahydrofolate and *V* values of the desensitized enzyme were 1.7 mM and 2.0 μmol of formaldehyde formed per hr/mg of protein, respectively.

Table 2. Serine transhydroxymethylase activity in L1210 solid tumors, in liver and kidney of normal and tumor-bearing mice, and in pig kidney

Tissue	Specific activity*
Pig kidney	0.012
Normal mice	
Liver	0.115
Kidney	0.017
L1210-tumor-bearing mice	
Liver	0.123
Kidney	0.017
L1210 solid tumor	0.098

* Micromoles of formaldehyde formed per hr/mg of protein.

Levels of Serine Transhydroxymethylase in Tissues of Pigs and Mice, and in L1210 Solid Tumors. It is evident from results presented in Table 2 that the specific activity in extracts obtained from the livers of both normal and tumor-bearing mice was higher than in both pig and mouse kidney. The level of activity in the L1210 tumors was high and was comparable to that present in the mouse liver.

Tetrahydrofolate Saturation Curve for Serine Transhydroxymethylase from L1210 Tumor. The interaction of tetrahydrofolate with serine transhydroxymethylase from L1210 tumors is shown in Fig. 2. The enzyme clearly followed hyperbolic saturation kinetics, as is evident from the linear double reciprocal plot. The Hill plot (insert Fig. 2) gave an *n* value of 1.

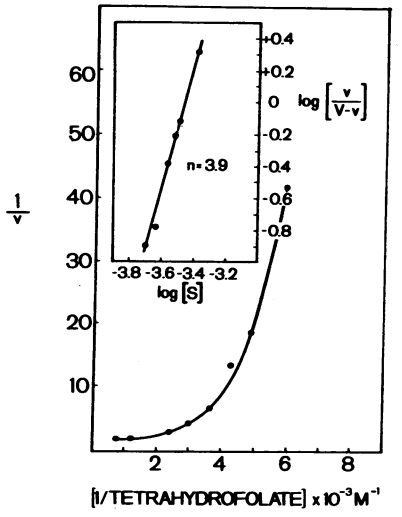

FIG. 1. Lineweaver–Burk plot for the saturation of pig kidney serine transhydroxymethylase by tetrahydrofolate. The purified enzyme (200 μg) was preincubated for 5 min at 37° with the tetrahydrofolate concentrations indicated in the figure. The reciprocal of the tetrahydrofolate concentration was plotted against the reciprocal of the initial velocity *v*, i.e., the μmol of formaldehyde formed per hr in the reaction mixture. The insert (Hill plot) represents the log of the tetrahydrofolate concentration plotted against the log of *v* divided by *V* the maximum velocity minus *v*. An *n* value of 3.9 was calculated from the slope of the plot.

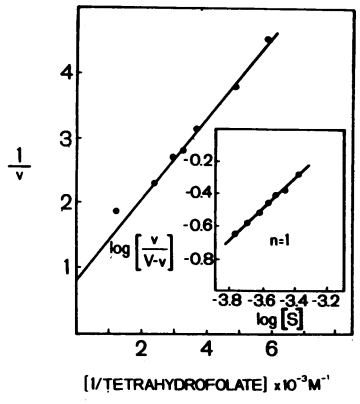

FIG. 2. Double reciprocal plots for the interaction of tetrahydrofolate with serine transhydroxymethylase from mouse L1210 tumor tissue. An aliquot of the supernatant fraction (0.1 ml containing 2.7 mg of protein) of L1210 tumor tissue was preincubated for 5 min at 37° with the tetrahydrofolate concentrations indicated in the figure and the enzyme activity was determined. An n value of 1 was calculated from the slope of the Hill plot (insert).

Saturation by Tetrahydrofolate of Serine Transhydroxymethylase from Normal and L1210-Tumor-Bearing Mice. The saturation by tetrahydrofolate of the enzyme from livers of normal and tumor-bearing mice is shown in Fig. 3. When the enzyme obtained from livers of normal mice was analyzed, the reciprocal plot curved upwards and the Hill plot (insert) gave an n value of 2.4. In contrast to these observations, serine transhydroxymethylase from liver of *tumor-bearing* animals gave a linear Lineweaver–Burk plot and an n value of 1. The saturation curves for tetrahydrofolate (v versus [S]) were hyperbolic in the case of the enzyme from livers of tumor-bearing animals and sigmoid for the enzyme from livers of non-tumor-bearing animals.

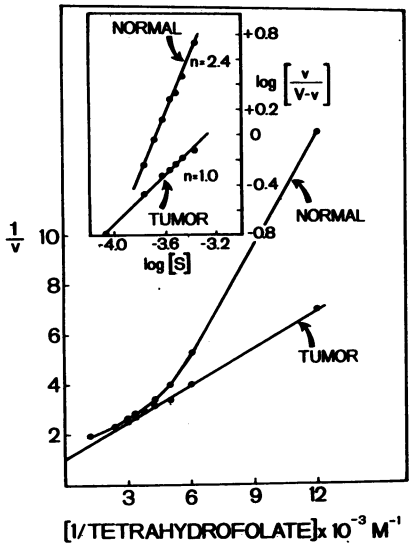

FIG. 3. Double reciprocal plots for the interaction of tetrahydrofolate with serine transhydroxymethylase from liver of mice bearing L1210 tumors and from normal mouse liver. Livers from normal mice and from mice bearing L1210 tumors were separately homogenized in 2 vol (wt/vol) of 0.3 M potassium phosphate buffer at pH 7.4 and centrifuged at 35,000 × g for 15 min. Aliquots of the supernatant fraction (0.1 ml) containing the enzyme (2.8 mg of protein the case of the normal mouse liver and 2.6 mg of protein for mouse liver from tumor-bearing animals) were separately preincubated for 5 min at 37° with the concentrations of tetrahydrofolate indicated in the figure and assayed for enzyme activity. An n value of 2.4 for the normal mouse liver enzyme and an n value of 1 for the mouse enzyme from tumor-bearing animals was calculated from the slope of the Hill plots.

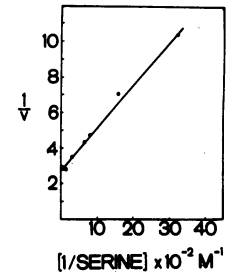

FIG. 4. Double reciprocal plot of the interaction of L-serine with purified pig kidney serine transhydroxymethylase. The purified enzyme (130 μg) was preincubated with the various concentrations of L-[2-^{14}C]serine indicated in the figure and 0.15 mM pyridoxal-P and assayed in the presence of 1.5 mM tetrahydrofolate. Identical results were obtained with the L1210 tumor and mouse liver enzyme preparations.

Identical results to those described above were obtained when kidney tissues from tumor-bearing and control animals were examined.

Saturation of Pig Kidney, L1210 Tumor, and Mouse Liver (Obtained from Normal and Tumor-Bearing Mice) Serine Transhydroxymethylase by L-Serine. When serine concentration was varied at a fixed concentration of tetrahydrofolate (1.5 mM), classical hyperbolic saturation kinetics (Fig. 4 represents saturation of the purified pig kidney enzyme) were observed and gave a K_m value of 0.9 mM, a V value of 2.7 μmol of formaldehyde formed per hr/mg of protein, and an n of 1 (Fig. 4).

DISCUSSION

An examination of the pathways for the interconversion of folate coenzymes reveals the central position occupied by 5,10-methylenetetrahydrofolate (17). *A priori* one would expect that enzymes involved in the synthesis and utilization of this key metabolite would be subject to modulation by effector molecules. Serine transhydroxymethylase catalyzes the formation of 5,10-methylenetetrahydrofolate from serine and tetrahydrofolate. The nonlinear effects of tetrahydrofolate on the enzyme–glycine complex (14) were interpreted to suggest cooperative interactions with tetrahydrofolate. Definitive evidence on the nature of these interactions was not presented.

The sigmoid saturation curve with tetrahydrofolate, nonlinear Lineweaver–Burk plot, and an n value of 3.9 strongly suggested the existence of positive cooperative effects (18) in the binding of tetrahydrofolate to the purified pig kidney serine transhydroxymethylase. This conclusion was further supported by the observation that denaturation by urea and renaturation by removal of urea resulted in desensitization of the enzyme to this allosteric interaction. One probable reason for earlier workers' not observing this phenomenon may have been that the heat denaturation step to remove contaminating proteins resulted in the desensitization of the enzyme to cooperative binding by tetrahydrofolate. The novel observation of cooperative interactions involving tetrahydrofolate with this polymeric protein suggests a probable regulatory role for this enzyme.

The level of the enzyme in L1210 solid tumors was about 10 times higher than in mouse or pig kidney. These elevated values were similar to those observed in livers of control and tumor-bearing (L1210) animals.

In contrast to the observation with the pig kidney enzyme,

the enzyme from L1210 solid tumors differed in that it exhibited Michaelis–Menten kinetics as evidenced by hyperbolic substrate saturation, linear reciprocal plots, and an n value of 1 (Fig. 2). An additional significant finding was that the enzyme from the liver and kidneys of normal mice was cooperative (Fig. 3), whereas the enzyme from tumor-bearing animals failed to show any cooperativity (Fig. 3) in its interactions with tetrahydrofolate. These results demonstrate that a significant change in the kinetic behavior of the liver and kidney enzyme has occurred due to the presence of the tumor. These results might be attributed to an alteration in enzyme structure resulting in the weakening of subunit interactions; *or* the production of a metabolite, possibly an activator; *or* the synthesis of a new enzyme; *or* the loss of an allosteric binding site.

The alteration in the kinetic properties of an enzyme present in tissues (liver and kidney) located distal to the site of a tumor indicates the possibility of the production of a chemical messenger by the tumor, which is transported to and produces biochemical changes in other tissues of the animal.

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