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Regulation of Monkey Liver Serine Hydroxymethyltransferase by Nicotinamide Nucleotide

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The positive homotropic binding of tetrahydrofolate to monkey liver serine hydroxymethyltransferase was abolished on preincubating the enzyme with NADH and NADPH. NAD^+ was a negative heterotropic effector, whereas NADP^+ was without effect. The allosteric effects of nicotinamide nucleotides on the serine hydroxymethyltransferase, reported for the first time, lead to a better understanding of the regulation of the metabolic interconversion of folate coenzymes.

Serine hydroxymethyltransferase (5,10-methylene-tetrahydrofolate–glycine hydroxymethyltransferase, EC 2.1.2.1) catalyses the reaction:

and could be considered as the first enzyme in the folate pathway. This reaction is the major source of one-carbon units in mammalian systems (Blakely, 1969). Regulation of serine hydroxymethyltransferase, which is positioned at a branch point in the pathway for the interconversion of folate coenzymes, should have a profound influence on cellular metabolism. Earlier attempts to demonstrate the regulation of this enzyme activity by nucleotides, folate coenzymes and intermediates as well as end products of purine and pyrimidine metabolism were unsuccessful (Rowe & Lewis, 1973).

The present paper reports the prevalence of co-operative interactions of H_4folate with the monkey liver serine hydroxymethyltransferase and the abolition of this co-operativity by reduced nicotinamide nucleotides. It is noteworthy that NAD^+ increased the sigmoidicity of the H_4folate -saturation curves, whereas NADP^+ had no effect.

Experimental

Materials

All biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., except the following. DL-[3- ^{14}C]Serine (specific radioactivity 48.5 mCi/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). H_4folate , prepared by the method of Hatefi *et al.* (1960), was kindly given

Abbreviations used: H_4folate , ($\pm$)-L-tetrahydrofolate; 5,10- $\text{CH}_2\text{-H}_4\text{folate}$, 5,10-methylenetetrahydrofolate; 5- $\text{CH}_3\text{-H}_4\text{folate}$, 5-methyltetrahydrofolate; 10- $\text{HCO-H}_4\text{folate}$, N^{10} -formyltetrahydrofolate; dimedone, 5,5-dimethylcyclohexane-1,3-dione.

by Dr. J. H. Mangum, Brigham Young University, Provo, UT, U.S.A. All other chemicals used were of analytical-reagent grade.

Animals

Adult bonnet monkeys (*Macaca radiata*), obtained from the Central Animal Facility, Indian Institute of Science, Bangalore, were used.

Methods

Assay of serine hydroxymethyltransferase. The procedure was essentially that described by Taylor & Weissbach (1965). Each 0.1 ml of assay mixture contained 400 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-2-mercaptoethanol, 10 mM-EDTA, 0.2 mM-pyridoxal 5'-phosphate, 1.8 mM- H_4folate , 1.8 mM-dithiothreitol and an appropriate amount of the enzyme (0.5–2.0 μg). The mixture was preincubated for 5 min at 37°C and the reaction started by the addition of 3.6 mM-L-[3- ^{14}C]serine (8.0×10^4 c.p.m.). After incubation for 15 min at 37°C, the reaction was stopped by the addition of 0.1 ml of dimedone (0.4 M in 50% ethanol). The mixtures were heated for 5 min at 100°C, cooled to 0°C and the [^{14}C]formaldehyde–dimedone adduct was extracted with 3 ml of toluene; a 1.0 ml portion of this extract in 5.0 ml of scintillation fluid (0.5% 2,5-diphenyloxazole in toluene) was counted for radioactivity in a Beckman LS-100 scintillation spectrometer. One unit of enzyme activity was defined as the amount that catalysed the formation of 1 μmol of formaldehyde/min at 37°C. Specific activity was expressed as units/mg of protein. Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Enzyme. Serine hydroxymethyltransferase from monkey liver (75 g) was purified by $(\text{NH}_4)_2\text{SO}_4$

fractionation (25–50% saturation), adsorption on CM-Sephadex C-50 and elution with a linear gradient of 0.05–0.5M-potassium phosphate buffer, pH 7.4, containing 1mM-2-mercaptoethanol, 20mM-EDTA and 0.05mM-pyridoxal 5'-phosphate. The pooled active fractions were freeze-dried, dissolved in 2.0ml of potassium phosphate buffer, pH 7.4, containing 0.05mM-pyridoxal 5'-phosphate, 20mM-EDTA and 1mM-2-mercaptoethanol and dialysed for 24h against two 50vol. changes of the same buffer. The dialysed enzyme was passed through a Sephadex G-200 column (82cm $\times$ 2.4cm) equilibrated with 0.1M-potassium phosphate buffer, pH 7.4, containing 1mM-2-mercaptoethanol, 20mM-EDTA and 0.05mM-pyridoxal 5'-phosphate. The active fractions were pooled and used as the enzyme in these studies.

Results

Co-operative interactions of H₄folate with monkey liver serine hydroxymethyltransferase

Fig. 1 shows the sigmoid saturation curve, when various concentrations of H₄folate were preincubated with the enzyme before starting the reaction with L-serine. The positive homotropic binding of H₄folate was also apparent from the *h* value of 2.5 calculated from the Hill plot (inset, Fig. 1). The substrate concentration at half-maximal velocity, *s*_{0.5} (H₄folate), was 0.9mM. Variation of the concentration of L-serine in the presence of saturating concentrations of H₄folate (result not given) shows a hyperbolic saturation of the enzyme. The *K_m* (L-serine) of 0.7mM was calculated from the Lineweaver–Burk plot.

On the other hand, when the enzyme was preincubated with L-serine, positive homotropic interaction of H₄folate with the enzyme was abolished (Fig. 1). The double-reciprocal plot was linear (inset, Fig. 2) and the Hill coefficient decreased from 2.5 in the absence of preincubation with L-serine, to 1.1 when preincubated with 3.6mM-L-serine. Concentrations of L-serine of less than 3.6mM partially abolished the co-operative interactions of H₄folate with the enzyme, as indicated by *h* values between 2.5 and 1.1 (not shown in Fig. 1). In these experiments the enzyme was preincubated with lower concentrations of L-serine, but was assayed at 3.6mM-L-serine.

Modulation of serine hydroxymethyltransferase activity by nicotinamide nucleotides

At low concentrations of H₄folate, NADH activated the monkey liver serine hydroxymethyltransferase (Fig. 2). Variation of the H₄folate concentration in the presence and absence of the activator showed that the positive homotropic

Fig. 1. H₄ folate saturation pattern of monkey liver serine hydroxymethyltransferase and the effect of serine on this saturation curve

The enzyme (2μg) was preincubated with H₄folate (concentrations indicated in the Figure) for 5 min at 37°C. The reaction was started by the addition of L-[3-¹⁴C]serine (3.6mM) and the assay mixtures were incubated for 15 min at 37°C. A duplicate set of reaction mixtures contained enzyme (2μg), which was preincubated with L-[3-¹⁴C]serine (3.6mM) for 5 min and the reaction was started by the addition of H₄folate (0.036–1.8mM). After incubation for 15 min at 37°C the [¹⁴C]formaldehyde formed was measured. ●, Preincubated with H₄folate; ○, preincubated with serine (3.6mM). The inset shows the Hill plot of the data obtained.

binding of H₄folate was abolished on adding the effector (Fig. 2). The double-reciprocal plot was linear (inset, Fig. 2) and an *h* value of 1.3 was obtained. The reduced nicotinamide nucleotide appears to activate the enzyme by decreasing the *s*_{0.5} (H₄folate), with no appreciable effect on the velocity at saturating concentrations of serine (3.6mM) and H₄folate (1.8mM). Variation of the concentration of NADH in the presence of a low concentration of H₄folate (result not given) shows a hyperbolic saturation of the enzyme with the activator. NAD⁺ (50mM) increased the positive co-operative interactions of H₄folate with the enzyme as shown by the increase in the Hill coefficient from *h* = 2.5 to *h* = 3.3 (Fig. 2). The non-linear double-reciprocal plot for H₄folate saturation in the presence of NAD⁺ is shown in the inset to Fig. 2.

NADPH also abolished the co-operative interactions of H₄folate with the monkey liver serine hydroxymethyltransferase (Fig. 3). Serially increasing concentrations of NADPH, from 5mM to 50mM,

Fig. 2. Heterotropic effect of NAD^+ and NADH on the H_4 folate saturation curve for monkey liver serine hydroxymethyltransferase

The saturation curve (●) for H_4 folate was obtained as described in Fig. 1 using $1.3 \mu\text{g}$ of the enzyme. The effects of NAD^+ (□) and NADH (○) were studied by incubating the enzyme already preincubated with H_4 folate (concentration indicated in the Figure) with NAD^+ (50mM) or NADH (10mM) for 5 min at 37°C . The reaction was started by the addition of $\text{L-[3-}^{14}\text{C]-serine}$ (3.6mM) and assayed as described under Methods. The presence of a high concentration of phosphate buffer served to nullify small changes in ionic strength produced by the addition of nucleotides. Care was taken to ensure that solutions of all reaction components were adjusted to pH 7.4. The Lineweaver-Burk plots in the absence of added nucleotides and in the presence of NAD^+ and NADH are shown in the inset. ●, No NADH ; ○, 10mM- NADH ; □, 50mM- NAD^+ .

shifted the sigmoid H_4 folate saturation curve to a hyperbolic one as reflected by a decrease in the h value from 2.5 in the absence of added NADPH to 1.1 at 50mM- NADPH . Enhancement of the enzyme activity by reduced nicotinamide nucleotides was marked at subsaturating concentrations of H_4 folate. No apparent effect was noticed at saturating concentrations of H_4 folate.

Discussion

The mechanism of action of serine hydroxymethyltransferase from rabbit liver (Akhtar *et al.*, 1975; Schirch *et al.*, 1977; Tatum *et al.*, 1977; Benkovic & Tatum, 1977), lamb liver (Ulevitch & Kallen, 1977a,b,c) has been extensively studied. Co-operative interactions of H_4 folate with serine hydroxymethyltransferase from mice liver and pig kidney was shown (Harish Kumar *et al.*, 1976). Rowe & Lewis (1973)

Fig. 3. Hill plots depicting the positive heterotropic effect of NADPH on the binding of tetrahydrofolate to monkey liver serine hydroxymethyltransferase

The Hill plot for H_4 folate saturation (●) was obtained as described in Fig. 1 using $1.4 \mu\text{g}$ of the enzyme. The Hill plots in the presence of NADPH (○, 5mM; ▲, 10mM; △, 50mM) were obtained by preincubating the enzyme with H_4 folate followed by incubation with NADPH . The velocity was determined as described under 'Methods'.

reported the absence of either activation or inhibition by non-folate compounds and folate analogues with the bovine liver enzyme. One reason for this observation could be the use of saturating concentrations of H_4 folate. The possible regulatory role of other enzymes, namely 5,10- CH_2 - H_4 folate reductase (5-methyltetrahydrofolate- NAD^+ oxidoreductase; EC 1.1.1.68; Kutzbach & Stokstad, 1967, 1971), 10- CHO-H_4 folate dehydrogenase (10-formyltetrahydrofolate- NADP^+ oxidoreductase; EC 1.5.1.6; Krebs *et al.*, 1976; Krebs & Hems, 1976) in one-carbon metabolism was suggested. However, no allosteric effects with the isolated enzymes were demonstrated.

The predominant folate coenzyme in the mammalian liver is 5- CH_3 - H_4 folate (Noronha & Silverman, 1962; Shin *et al.*, 1972). As the concentration of

H₄folate required to obtain measurable activity is greater than 0.2mM (Fig. 1), this form of the coenzyme should have been present in appreciable amounts in the liver tissue. The positive heterotropic effect of reduced nicotinamide nucleotide resulted in the marked enhancement of the enzyme activity at low concentrations of H₄folate [e.g. about 15-fold at 0.2mM-H₄ folate and 2-fold at $s_{0.5}$ (H₄folate)]. This observation, coupled to the essentially irreversible nature of the reaction catalysed by 5,10-CH₂-H₄-folate reductase, could explain the occurrence of 5-CH₃-H₄folate as the major folate coenzyme form in the liver.

Modulation of serine hydroxymethyltransferase activity by metabolites could have a profound influence on the availability of one-carbon fragments for a variety of biosynthetic reactions.

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References

- Akhtar, M., El-Obeid, H. A. & Jordan, P. M. (1975) *Biochem. J.* **145**, 159–168
- Benkovic, S. J. & Tatum, C. M., Jr. (1977) *Trends Biochem. Sci.* **2**, 161–163
- Blakely, R. L. (1969) in *The Biochemistry of Folic Acid and Related Pteridines*, North-Holland Publishing Co., Amsterdam and London
- Harish Kumar, P. M., North, J. A., Mangum, J. H. & Appaji Rao, N. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1950–1953
- Hatefi, Y., Talbert, P. T., Osborn, M. J. & Huennekens, F. M. (1960) *Biochem. Prep.* **7**, 89–92
- Krebs, H. A. & Hems, R. (1976) *Adv. Enzyme Regul.* **14**, 493–513
- Krebs, H. A., Hems, R. & Tyler, B. (1976) *Biochem. J.* **158**, 341–353
- Kutzbach, C. A. & Stokstad, E. L. R. (1967) *Biochim. Biophys. Acta* **139**, 217–220
- Kutzbach, C. & Stokstad, E. L. R. (1971) *Biochim. Biophys. Acta* **250**, 459–477
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Noronha, J. M. & Silverman, M. (1962) *J. Biol. Chem.* **237**, 3299–3302
- Rowe, P. B. & Lewis, G. P. (1973) *Biochemistry* **12**, 1962–1969
- Schirch, L., Tatum, C. M., Jr. & Benkovic, S. J. (1977) *Biochemistry* **16**, 410–419
- Segel, I. H. (1975) *Enzyme Kinetics*, pp. 371–375, John Wiley and Sons, New York
- Shin, Y. S., Williams, M. A. & Stokstad, E. L. R. (1972) *Biochem. Biophys. Res. Commun.* **47**, 35–43.
- Tatum, C. M., Jr., Benkovic, P. A., Benkovic, S. J., Potts, R., Schleicher, E. & Floss, H. G. (1977) *Biochemistry* **16**, 1093–1102
- Taylor, R. T. & Weissbach, H. (1965) *Anal. Biochem.* **13**, 80–84
- Ulevitch, R. J. & Kallen, R. G. (1977a) *Biochemistry* **16**, 5342–5349
- Ulevitch, R. J. & Kallen, R. G. (1977b) *Biochemistry* **16**, 5350–5354
- Ulevitch, R. J. & Kallen, R. G. (1977c) *Biochemistry* **16**, 5355–5363