Ann. Rev. Nutr. 1985. 5:115–41

VITAMIN B_{12}–FOLATE INTERRELATIONSHIPS

Barry Shane

Department of Biochemistry, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, Maryland 21205

E. L. Robert Stokstad

Department of Nutritional Sciences, University of California, Berkeley, California 94720

CONTENTS

0199-9885/85/0715-0115$02.00

BACKGROUND

The interrelationship between vitamin B_{12} and folate metabolism in man is best illustrated by the hematologically indistinguishable, macrocytic megaloblastic anemia resulting from a deficiency of either vitamin. Large pharmacological doses of either vitamin will elicit a hematological response in patients suffering from a deficiency of either or even both vitamins (61, 119). Large doses of folate cause a temporary or partial hematological remission in pernicious anemia patients but fail to correct the neurological lesions that arise from prolonged vitamin B_{12} deprivation. The relationship can also be demonstrated biochemically in man and in experimental animals by the common deficiency symptoms of elevated urinary excretion of formiminoglutamate, aminoimidazolecarboxamide, and formate, all of which indicate a primary defect in folate metabolism. Methionine is also involved in this interrelationship, as its administration normalizes many of the biochemical indicators of folate deficiency in vitamin B_{12} deficiency in man and experimental animals. However, methionine exacerbates the megaloblastic changes in the bone marrow of vitamin B_{12}–deficient patients.

The interrelationships among folate, vitamin B_{12}, and methionine metabolism have been the subject of several reviews (22, 40, 90, 93, 100). This review updates recent information on the metabolic relationships involved. A brief background on those areas of folate metabolism that bear directly on the subject is presented.

FOLATE METABOLISM

Folate coenzymes serve as acceptors or donors of one-carbon units in a variety of reactions involved in amino acid and nucleotide metabolism. Some of these reactions, known as one-carbon metabolism, are shown in Figure 1. The coenzyme forms of the vitamin are the tetrahydro derivatives (Figure 2). These can accept one-carbon units at the oxidation level of formate (from formiminoglutamate, a histidine catabolite, or formate) and at the level of formaldehyde (from serine). Formate, in the form of 10-formyl-H_4PteGlu[1], is utilized in the de novo biosynthesis of the purine ring, while formaldehyde, in the form of 5,10-methylene-H_4PteGlu, is utilized in the synthesis of thymidylate from deoxyuridylate. 5,10-Methylene-H_4PteGlu, which is freely interconvertible with 5,10-methenyl- and 10-formyl-H_4PteGlu, can also be reduced to 5-methyl-H_4PteGlu. The methyl group of this compound is used in the biosynthesis of methionine from homocysteine. Figure 1 depicts the interconversion and

[1]Abbreviations used: PteGlu, pteroylglutamic acid, folic acid; H_4PteGlu$_n$, tetrahydropteroyl-poly-γ-glutamate, where n indicates the number of glutamate residues.

metabolism of pteroylmonoglutamates. However, practically all the folates in mammalian tissues, with the exception of plasma, are present as conjugated folylpolyglutamate derivatives (Figure 2). Most, if not all, of the reactions outlined would use these polyglutamate forms as substrates under physiological conditions.

Amino Acid Interconversions

Serine hydroxymethyltransferase, a pyridoxal phosphate-containing enzyme, catalyzes the reversible transfer of formaldehyde from serine to H_4PteGlu (Figure 1, reaction 3) to generate 5,10-methylene-H_4PteGlu and glycine. In mammalian tissues the β-carbon of serine is the major source of one-carbon units for folate metabolism.

5,10-Methylene-H_4PteGlu can be metabolized in a number of directions. A major pathway in mammalian tissues involves its reduction to 5-methyl-H_4PteGlu (Figure 1, reaction 10) followed by the transfer of the methyl group to homocysteine to form methionine and regenerate H_4PteGlu (Figure 1, reaction 11). The reduction of 5,10-methylene-H_4PteGlu is catalyzed by the flavoprotein methylenetetrahydrofolate reductase and NADPH is required to reduce enzyme-bound FAD. The reaction is essentially irreversible under physiological conditions, making it the first committed step in methionine biosynthesis. Methionine exerts feedback control over the reaction via adenosylmethionine inhibition of the reductase (55, 111).

Methionine synthetase catalyzes the transfer of the methyl group from 5-methyl-H_4PteGlu to homocysteine to form methionine. The mammalian enzyme contains tightly bound cobalamin, which is methylated by the folate substrate. The methyl group is then transferred from methylcobalamin to homocysteine to generate methionine (106). This is the only reaction known in mammalian tissues for the metabolism of 5-methyl-H_4PteGlu with the subsequent regeneration of H_4PteGlu. Adenosylmethionine and a reducing system are required in vitro for an initial priming of the enzyme-bound cobalamin. Whether adenosylmethionine is required in vivo to methylate the cobalamin has not been established.

Methionine synthetase is one of three mammalian enzymes known to require vitamin B_{12} as a cofactor, the others being methylmalonyl-CoA mutase, which contains bound 5-deoxyadenosylcobalamin, and leucine 2,3-aminomutase (78). Although a B_{12}-independent enzyme was reported in mammalian tissues, more recent studies aimed at detecting the B_{12}-independent activity have not been successful (17). The methionine synthetase reaction is subject to inhibition by methionine although methionine is only a weak inhibitor of the mammalian enzyme.

Folate is also involved in the metabolism of formiminoglutamate, a histidine catabolite (Figure 1, reaction 12). Formiminotransferase catalyzes the transfer

of the formimino group to H_4PteGlu to generate 5-formimino-H_4PteGlu and glutamate. The formimino group is at the oxidation level of formate. Formimino-H_4PteGlu is metabolized by deamination to 5,10-methenyl-H_4PteGlu (Figure 1, reaction 13) in a reaction catalyzed by a cyclodeaminase. In mammalian tissues, formiminotransferase and cyclodeaminase activities are associated with a single polypeptide (59). Under conditions of folate deficiency, formiminoglutamate catabolism is impaired and it is excreted in elevated amounts by experimental animals and humans.

Thymidylate Synthesis

Although one-carbon metabolism is not involved in the de novo synthesis of pyrimidines, folate is required for the synthesis of thymidylate (Figure 1, reaction 9). The reaction is catalyzed by thymidylate synthetase, and involves the transfer of formaldehyde to the 5-position of deoxyuridylate. The pyrazine ring of H_4PteGlu supplies the reducing component for the reduction of the transferred formaldehyde to methanol, which results in the oxidation of H_4PteGlu to H_2PteGlu. The formation of deoxynucleotides, mediated by thymidylate synthetase and ribonucleotide reductase, is considered to be the rate-limiting step in DNA synthesis. Mammalian cells can also synthesize thymidylate via the thymidine kinase–mediated salvage pathway.

H_2PteGlu formed in the thymidylate synthetase reaction is functionally

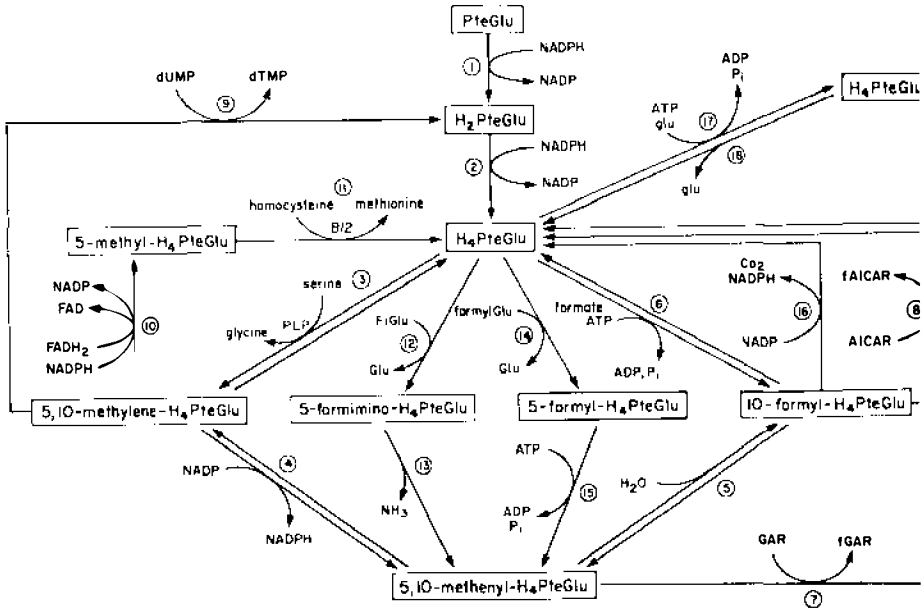

Figure 1 Metabolic reactions of one-carbon metabolism in the mammalian cell cytoplasm.

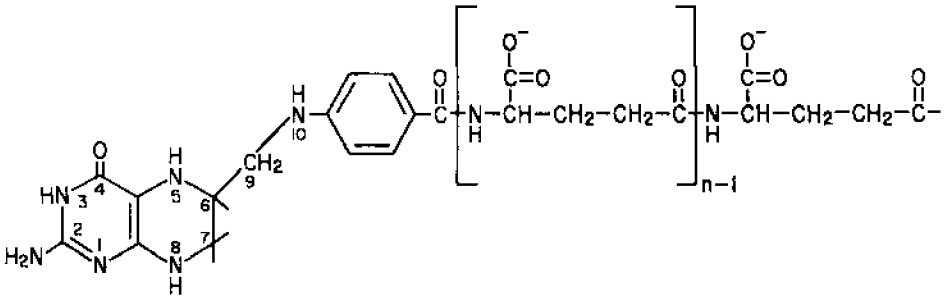

Figure 2 Tetrahydropteroylpoly-γ-glutamate ($H_4PteGlu_n$).

inactive as a coenzyme and has to be reduced to $H_4PteGlu$ before it can participate in one-carbon transfer reactions (Figure 1, reaction 2). This reduction is catalyzed by dihydrofolate reductase, an enzyme that also catalyzes the reduction of PteGlu. Normally, PteGlu is not found in unsupplemented foods and the major role of dihydrofolate reductase appears to be to reduce $H_2PteGlu$ formed in the thymidylate synthetase reaction.

Purine Biosynthesis

The C-8 and C-2 positions of the purine ring are derived from the one-carbon pool (Figure 1, reactions 7, 8) in reactions catalyzed by glycinamide ribonucleotide (GAR) transformylase and 5-amino-4-imidazolecarboxamide ribonucleotide (AICAR) transformylase, respectively. 10-Formyl-$H_4PteGlu$ is the one-carbon donor for both reactions (96).

FOLYLPOLYGLUTAMATES AND FOLATE HOMEOSTASIS

The role of folylpolyglutamates was the subject of several recent reviews (21, 50, 66). Folylpolyglutamates are the major intracellular forms of the vitamin and are the natural substrates for the enzymes of one-carbon metabolism. They are as effective as, and in some cases more effective than, pteroylmonoglutamates as substrates for the enzymes of one-carbon metabolism.

Some of the enzymes of one-carbon metabolism (Figure 1) are present as multifunctional proteins in mammalian tissues. Substrate channelling with polyglutamate substrates has been observed for the bifunctional protein formiminotransferase-cyclodeaminase (60) without release of the intermediate product. This phenomenon, which is not observed with the monoglutamate substrate, increases the local concentration of the intermediate 5-formimino-$H_4PteGlu$ product and also prevents the accumulation of this nonfunctional intermediate. Folylpolyglutamates may also play an important role in the regulation of one-carbon metabolism. Recent studies show that they effectively

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.