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Review

Folates: Chemistry, analysis, occurrence, biofortification and bioavailability

Ramesh Kumar Saini *, Shivraj Hariram Nile, Young-Soo Keum *

Department of Bioresources and Food Science, College of Life and Environmental Sciences, Konkuk University, Seoul 143-701, Republic of Korea

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ABSTRACT

Folates (Vitamin B_9) include both naturally occurring folates and synthetic folic acid used in fortified foods and dietary supplements. Folate deficiency causes severe abnormalities in one-carbon metabolism can result chronic diseases and developmental disorders, including neural tube defects. Mammalian cells cannot synthesize folates *de novo*; therefore, diet and dietary supplements are the only way to attain daily folate requirements. In the last decade, significant advancements have been made to enhance the folate content of rice, tomato, common bean and lettuce by using genetic engineering approaches. Strategies have been developed to improve the stability of folate pool in plants. Folate deglutamylation through food processing and thermal treatment has the potential to enhance the bioavailability of folate. This review highlights the recent developments in biosynthesis, composition, bioavailability, enhanced production by elicitation and metabolic engineering, and methods of analysis of folate in food. Additionally, future perspectives in this context are identified. Detailed knowledge of folate biosynthesis, degradation and salvage are the prime requirements to efficiently engineer the plants for the enhancement of overall folate content. Similarly, consumption of a folate-rich diet with enhanced bioavailability is the best way to maintain optimum folate levels in the body.

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1. Introduction

Vitamin B_9, also known as folate or folic acid (pteroyl-L-glutamic acid) is the fully oxidized monoglutamate form of the folate vitamin that is synthetically produced and used in fortified foods and dietary supplements (Crider, Bailey, & Berry, 2011). In the body, folate is converted to dihydrofolate, tetrahydrofolate, L-methyl folate and other derivatives, which participate in specified biological activities (Greenberg, Bell, Guan, & Yu, 2011). Folate plays an essential role in association with B_{12} and B_6 vitamins, mainly in nucleotide synthesis, methionine regeneration from homocysteine, and oxidation and reduction of one-carbon units required for normal cell division and growth (Crider, Yang, Berry, & Bailey, 2012; Fekete et al., 2012; McGarel, Pentieva, Strain, & McNulty, 2015). An important folate derivative, *i.e.* 5-methyltetrahydrofolate

* Corresponding authors.
E-mail addresses: saini_1997@yahoo.com (R.K. Saini), rational@konkuk.ac.kr (Y.-S. Keum).

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($5CH_3$-THF; $5CH_3$-H_4folate) plays a key role as a methyl donor in the regeneration of methionine from homocysteine (Hcy), produced in the methylation cycle of one carbon metabolism (Fox & Stover, 2008). Methylation reactions (epigenetic modification) are important mechanisms of gene regulation in eukaryotic cells. The degree of methylation of DNA varies among tissues and changes during development; generally, hypermethylation of DNA prevents transcription (Smith & Meissner, 2013).

Folate and vitamin B_{12} deficiency causes severe abnormalities in one-carbon metabolism, which is considered a risk factor for some chronic diseases and developmental disorders, including autism (Lyall, Schmidt, & Hertz-Picciotto, 2014), Alzheimer's disease (Hinterberger & Fischer, 2013), senile dementia (mental deterioration in old age) (Araújo, Martel, Borges, Araújo, & Keating, 2015), and neural tube defects (NDTs) (Copp, Stanier, & Greene, 2013). NTDs are a group of abnormalities of the brain, spine and spinal cord which typically manifest during the first month gestation period, causing the failure of the neural tube to close during embryogenesis (Williams et al., 2015). Thus, a folate-rich diet and a folic acid supplement are strongly recommended during pregnancy to prevent NTDs, and other chronic dysfunctions, such as congenital heart defects (CHDs) (Czeizel, Dudás, Vereczkey, & Bánhidy, 2013).

Dark green leafy vegetables, fruits, grains, legumes and dairy products are the major source of dietary folate (USDA, 2016). Dairy (*e.g.*, Milk fermented with folate-producing starter cultures) and animal-derived products (*e.g.*, Egg white nanoparticles as folate carriers) with enhanced physical and biological functionalities are also emerging as a functional ingredient to improve folate status in animals (Arzeni, Pérez, LeBlanc, & Pilosof, 2015; Laiño, Zelaya, Juárez del Valle, Savoy de Giori, & LeBlanc, 2015). Though fortified foods with synthetic folic acid are the primary source of dietary folate in developed countries, possible adverse effects of synthetic folic acid, such as masking symptoms of vitamin B_{12} deficiency and prostate cancer risk have raised the health concerns on synthetic folic acid supplementation (Wien et al., 2012). Hence, foods rich in natural folates such as fruits and vegetables have received much attention all over the world. Thus, increasing the consumption of folate-rich fruits and vegetable with enhanced bioavailability is the best way to combat folate deficiency complications without health concerns (Lee & Chan, 2011).

In the last decade, great advancements have been made to enhance the folate content of rice, tomato, common bean and lettuce by using genetic engineering approaches. Strategies have been developed to improve the stability of folate pools in plants through silencing of folate converting enzymes. Folate deglutamylation through food processing and thermal treatment has been shown to increase the bioavailability, as folate can be absorbed only in monoglutamate form. Thus, this review discusses the recent developments in biosynthesis, composition, bioavailability, enhancement, and methods of analysis of folate in food. Additionally, future research challenges in this context are also identified.

2. Structure and biosynthesis of folate

Folic acid consists of a p-aminobenzoate (p-ABA) molecule linked to a pteridine ring and a molecule of glutamic acid (Fig. 1a). Food folates, which exist in diverse forms, contain additional glutamate residues, making them polyglutamate. Various forms of folate differ in one carbon unit linked at N_5- and/or N_{10}-position of the pteridine ring (Table 1), such as methyl (5-CH_3), methylene ($5,10$-CH_2), formimino (5-CHNH), formyl (5- or 10-HCO), and methenyl (5,10-CH). During folate biosynthesis in higher plants, pteridine, p-ABA and glutamate (Glu) moieties are synthesized in plastids, mitochondria, and chloroplast, respectively (Hanson & Gregory, 2011). A detailed pathway of folate biosynthesis is illustrated in Fig. 1b. Biosynthesis of pteridine moieties (Fig. 1b, steps 1 to 4) begins in the cytosol with the hydrolysis of guanosine triphosphate (GTP) to form 7,8-dihydroneopterin 3′-triphosphate (DHN-P_3), which is mediated by GTP cyclohydrolase I (GTPCHI). The DHN-P_3

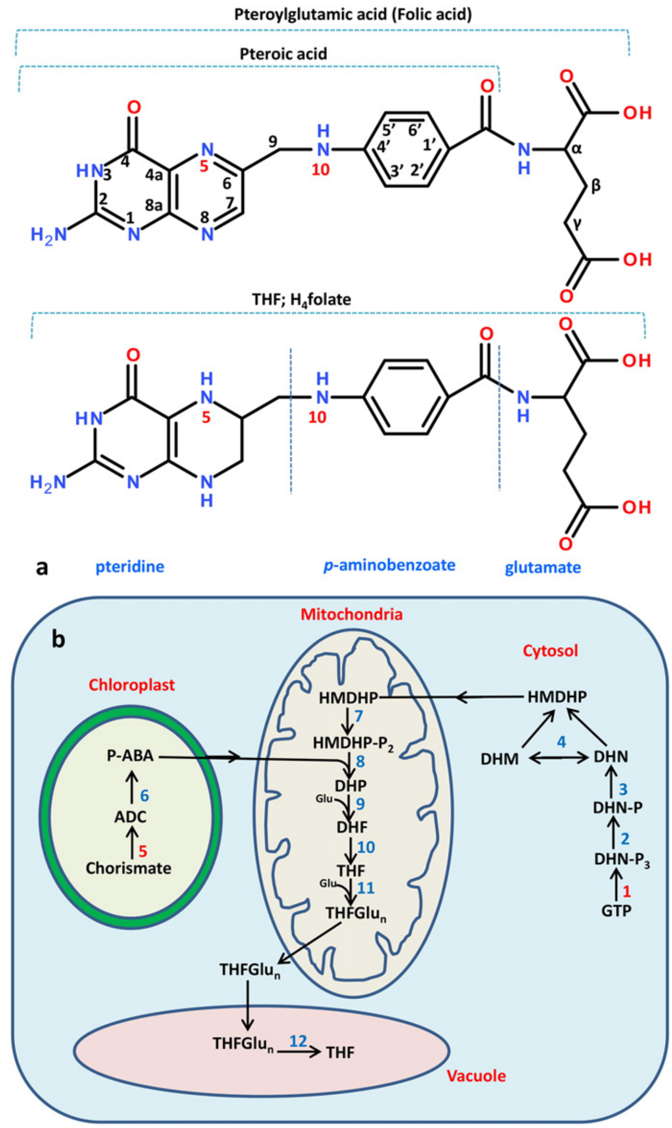

Fig. 1. a) Chemical structure of folic acid and H_4folate (mono glutamyl form), all these three moieties of H_4folate are produced separately in plastids, mitochondria, and chloroplast. B) Folate biosynthesis pathway, its compartmentalization in plant cells (Hanson & Gregory, 2011; Saini, 2013). Abbreviation of enzymes: 1. GTP cyclohydrolase (GTPCHI), 2. DHN-P_3 pyrophosphatase, 3. Non-specific phosphatase, 4. Dihydroneopterin aldolase, 5. Aminodeoxychorismate synthase (ADCS), 6. Aminodeoxychorismate lyase, 7. Hydroxymethyldihydropterin pyrophosphokinase, 8. Dihydropteroate synthase, 9. Dihydrofolate synthase, 10. Dihydrofolate reductase, 11. Folylpolyglutamate synthase, and 12. γ-glutamyl hydrolase (GGH). Abbreviations of compounds: ADC - Aminodeoxychorismate, DHF - Dihydrofolate, DHM - dihydromonapterin, DHN - Dihydroneopterin, DHP - Dihydropteroate, –Glc - Glucose ester, –Glu$_n$ - Polyglutamate, HMDHP - Hydroxymethyldihydropterin, –P - Phosphate, –P_2 - Diphosphate, –P_3 - Triphosphate, THF- Tetrahydrofolate.

Table 1
Substituent groups and their positions in common folate forms.

Name	Abbreviations	Position N_5	Position N_{10}
Pteroylglutamic acid	Folic acid	–	—H
7,8-Dihydrofolate	H_2folate	—H	—H
5-Methyl-5,6-dihydrofolate	5-CH_3-H_2folate	—CH_3	—H
5,6,7,8-Tetrahydrofolate	H_4folate	—H	—H
5-Methyltetrahydrofolate	5-CH_3-H_4folate	—CH_3	—H
5-Formyltetrahydrofolate	5-CHO-H_4folate	—CHO	—H
10-Formyltetrahydrofolate	10-CHO-H_4folate	—H	—CHO

Source- (Eitenmiller et al., 2007).