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Physico-Chemical Characteristics, Analytics and Metabolism of Folate in Plants

Ashok Verma K[1,3]*, Akhilesh Pandey K[1] and Neeraj Dubey K[2]

[1]Department of Biochemistry, Banaras Hindu University, Varanasi, India
[2]Department of Plant Pathology and Weed Research, ARO, Newe-Yaar Research Center, Ramat Yishay, Israel
[3]Sugarcane Research Institute, Shahjahanpur, India

Abstract

In one carbon reaction, tetrahydrofolate works as cofactor for the synthesis of glycine, serine, methionine, purines and thymidylate. Folate or vitamin B9 is not synthesized in humans therefore plants are the main source of this essential vitamin. Deficiency of vitamin B9 may cause severe health consequences like birth defects, megaloblastic anemia, cardiovascular disorders, cancers, etc. To recover folate intake worldwide in the daily diet it is essential to engineer genetically universal food plants with intrinsic capacity to synthesize folate. In this review, we have precised recent advances in determination, biosynthesis enzymes, transport and compartmentation of folate.

Keywords: Folate; Chemical analysis; Biosynthesis; Plant metabolism; Compartmentation; Transport

Abbreviations: THF: Tetrahydrofolate; PABA: Para-Aminobenzoic Acid; HPLC: High Performance Liquid Chromatography; GTPCHI: Guanosine Triphosphate Cyclohydrolase I; DHNTPDP: Dihydroneopterin Triphosphate Diphosphatase; DHNA: Dihydroneopterin Aldolase; ADCS: Aminodeoxychorismate Synthase; ADCL: Aminodeoxychorismate Lyase; HMDHP: 6-Hydroxymethyldihydropterin Pyrophosphokinase; DHPS: Dihydropteroate Synthase; DHFS: Dihydrofolate Synthetase; DHFR: Dihydrofolate Reductase; FPGS: Folylpolyglutamate Synthase; FBP: Folate Binding Protein; PGA: Pteroylglutamic Acid; LC-MS: Liquid Chromatography

Introduction

Folates play a vital role in human beings and they involves in extensive range of biochemical pathways. Folates represent a group of water soluble vitamins B and its levels vary in food plants. In general, they extensively found in yeast, green leaves of vegetables, egg yolk and liver of animals. Folates are components of enlarged family of polyglutamates (5-7 glutamyl residues) of pteroic acid and related analogs. They are crucial co-factors in one carbon transfer reactions for biosynthesis of purines, pyrimidines, formylmethionyl-tRNA and thymidylate and in the metabolism of several amino acids such as methionine, serine and glycine [1]. Tetrahydrofolate synthesis occurs only in plants, fungi, microbes and parasites. In humans and other animals it is absent because of lacking of complex biochemical pathways and important enzymes. The first step of folates biosynthesis in plants comprised with synthesis of pterin precursors from GTP in the cytosol (pterin branch), and p-aminobenzoate (PABA) from chorismate in plastids (PABA branch). The biosynthesis of pterins are controlled by GTP-cyclohydrolase I (GTPCHI; EC 3.5.4.16), this is a rate limiting step. In another step, both pterin precursors and PABA are introduced into the mitochondria to take part in the condensation to folates [2].

In human beings, the main sources of folates are plant and it needs to be consumed a minimum of 400 µg/day through the diet [3]. In developing and developed countries, it is a fact for marked folate deficiency in extent of population, even in the regions where folate supplementation of foods is in practice and in the regions where level of intake of folates is in excess of the recommended dietary allowance [4]. Folates deficiency in the daily diet causes decrease in DNA synthesis and disturb to usual rate cell division. It is also associated with reduction in the biosynthesis of cells in the bone marrow results in the development of megaloblastic anemia, neural tube defects in infants (such as spina bifida and anencephaly), increased risk of vascular disease and certain types of cancer [5]. In addition to these diseases, lower serum folate levels have been also observed in patients with Alzheimer disease and vascular dementia [6] Folic acid supplements as multivitamin tablets guided remarkable decrease in neural tube defects [7]. Under controlled condition, application of some folic acid fortified grain products also declined in the occurrence of neural tube defects [8], childhood cancer [9] and stroke [10]. The genetic engineering approaches for folate biofortification of food grains come into view to be a cost effective and sustainable balancing approach to the existing interference (industrial fortification, folate pill distribution and diet diversification), predominantly for rural population in the developing countries, where conventional approaches due to high recurrent costs are difficult to apply.

Chemical properties of folates

Chemical compounds with similar chemical properties and biological activity to folic acid are recognized as folate. The basic structure of folate comprises of 2-amino-4-hydroxy-6-methylpterin (pteridine ring) linked through a methylene bridge to para-aminobenzoate which is conjugated with one or several L-glutamic acid residues with γ-peptide linkage (Figures 1A and 1B).

The compounds of identical chemical properties and analogous metabolic activities within the same vitamin family may be called vitamers [11]. The structure of folate exhibits dissimilarities at three sites, as a result large number of chemically linked species. Firstly, the pteridine ring can be fully oxidized (as in folic acid), partially reduced at the 7, 8-position (H_2 folate), or can get fully reduced.

***Corresponding author:** Ashok Verma K, Department of Biochemistry, Banaras Hindu University, Varanasi, India, Tel: 0542-2307539; E-mail: vrmashok75@gmail.com

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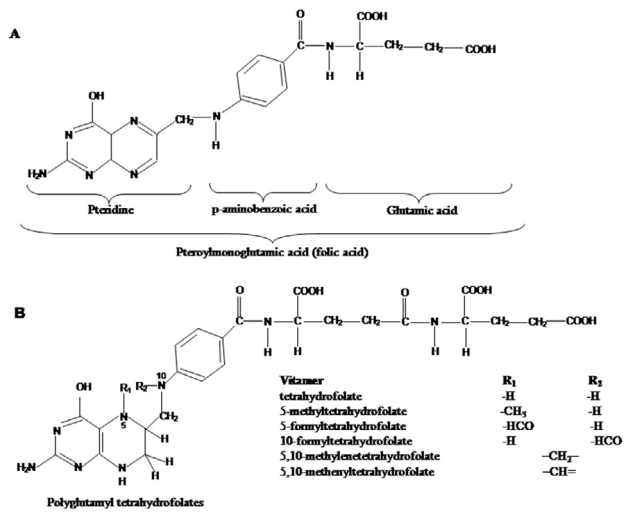

Figure 1: Chemical structure of (A) folic acid and (B) polyglutamyl tetrahydrofolate and its C_1 substituted derivatives. Natural folates occur mainly as polyglutamylated forms in which a γ-linked chain of up to about eight residues is added to the glutamate moiety.

According to the recommendations of IUPAC, the reduced 5, 6, 7, 8-tetrahydropteroylglutamic acid is called tetrahydrofolate and is abbreviated as H_4 folate [12]. Secondly, tetrahydrofolate can be substituted at the N_5 or N_{10} position to form 5-methyl, 5-formyl, 5-formimino, and 10-formyl derivatives. Additionally, N_5 and N_{10} can be bridged to form 5, 10-methylene and 5, 10-methenyl derivatives. Thirdly, the big difference occurs in number of glutamyl residues. In the cell, folates are usually found in the form of folylpolyglutamates and act as the essential coenzyme, they cannot pass easily through cell membrane. Folylpolyglutamates are chemically very active, they show higher binding affinity with many other enzymes as compared to monoglutamates however, the length of glutamyl side chain adjust the flux of one-carbon units in different biochemical reactions [13]. The fully reduced folates contain two chiral centers, α-C atom in the glutamyl moiety and C_6 atom in the pteridine moiety. The natural diastereoisomeres of H_4 folate, 5-CH_3-H_4 folate, and 5-HCO-H_4 folate are [6S, αS] diastereoisomeres, whereas the naturally occurring forms of 10-HCO-H_4 folate, 5, 10-CH_2-H_4 folate, and 5, 10-CH^+-H_4 folate are [6R, αS] diastereoisomeres. Most of the folates are light sensitive and oxidized readily. Oxidation process damage to folates and it is very high in reduced folates as compared to folic acid. The oxidation of folates is used in the pharmaceutical industry and for fortification [14]. The stability of folates depend on substitution of N positions, it is found more on N_5 or N_{10} positions and the stability order in aqueous solutions is 5-$HCOH_4$ folate > 5-CH_3-H_4 folate > 10-HCO-H_4 folate > H_4 folate [15]. The oxidation folates degrades it into biologically inactive compounds and the rate of degradation depends on pH, temperature, types of buffer, and the presence of catalysts or antioxidants/reducing agents [16,17]. The pH dependent inter-conversion between different folates forms is possible and it makes them stable to oxidation degradation for instance, 10-$HCOH_4$ folate is highly prone to oxidation and is easily converted to 10-HCO-PGA and 10-$HCOH_2$ folate while 5-CH_3H_4 folate gets oxidized to 5-CH_3H_2 folate [18]. In mild acidic pH of 5, 10-CH_2H_4 folate gets readily dissociated to H_4 folate and in acidic pH it converted into 5-$HCOH_4$ folate, 10-$HCOH_4$ folate and 5, 10-CH^+H_4 folate [19]. Although several factors affecting folates stability have been recognized, our knowledge related to stability of folates in complex systems such as foods is still limited.

Analytical advances

For analysis of folates, an appropriate method needs to be address because this is very challenging in plant samples. Folates exist in different forms and stabilities and their concentrations occur comparatively low in various biological materials. They are sensitive to heat, light, and oxygen. Many biological materials contain other enzymes and endogenous conjugates which cause inter-conversion between vitamers and changes in vitamer distribution. Presently, the most common techniques of folates analysis in food are microbiological assays, ligand binding assays, and high-performance liquid chromatographic (HPLC) methods. Nevertheless, before to actual measurement of folates, many steps require special attention like extractions of folates from the samples, enzyme treatments, purification and ensuring folates stability during the storage of samples. For standardizing methods of extraction and analysis of folates, the certified reference materials are highly recommended according to an inter-laboratory evaluation [20].

Sample extraction and enzyme treatments

Folates extraction from the food matrix is achieved by heat treatment in reducing agents containing buffers. The pH of the buffer is maintained in the range of neutral or mildly acidic or alkaline. A dry matter basis, a buffer to sample ratio at least ten is used during extraction. For reducing agent ascorbic acid is used very commonly. To avoid formaldehyde formation from ascorbate, the use of 2-mercaptoethanol with ascorbate was recommended by Wilson & Horne, [21]. With formaldehyde mercaptoethanol can form hemithioacetal and these blocks inter-conversion of folates. In extraction, heat treatment is applied to liberate of folates from the food matrix and folate binding proteins [22]. Heat treatment is not needed if tri-enzyme treatment is applied in the extraction samples however, this process may not be appropriate for all types of folates analyses [23]. In food, majority of folates occur in polyglutamate form and in traces mono and diglutamates form. To determine these monoglutamates, diglutamates and polyglutamates need to be hydolyzed by using 30 conjugates (γ-glutamylhydrolases; EC 3.4.22.12). In present analytical methods the most common conjugates with different pH optima being use are human and rat plasma, chicken pancreas, and hog kidney. The most common used conjugates are chicken pancreas suitable for microbiological assays it generates diglutamates, rat serum is suitable for HPLC analysis of monoglutamyl and hog kidney produces monoglutamates, involves in extraction and purification of folates.

Microbiological assay

This method is considered as a time consuming and needed special skills [24]. However, some changes have immensely added to the practicability of this method like cryo-protection in glycerol, reduce the time needed for the assay and increase the reproducibility [25]. The use of microtiter plates instead of test tubes increases the sample throughput, lowers the detection limit and reduces time and effort [26] whereas use of chloramphenicol resistant strain has reduced the need for aseptic working conditions [27]. Microbiological assays are based on nutrient (folates) requirements microorganisms in nutrient culture media. The growth of the microorganism depends on concentration of the folates in the sample and it measured turbidimetrically. In folates analysis, Lactobacillus rhamnosus ATCC 7469 microorganism is used most commonly. The microorganism L. rhamnosus ATCC

7469 frequently used in analysis of mono, di, and triglutamates [28]. Therefore, enzymatic deconjugation of long chain folylpolyglutamates is essential. The microorganism may be compatible for the individual folates and they may exhibit a considerable different response to different vitamers [29]. This is a 'reference' or 'gold standard' method and used frequently in the validation of analytical methods measuring individual vitamers. This method provides equal response to different vitamers however, there is a possibility that the growth of the microorganism is either enhanced or inhibited by non-folate compounds in the sample.

Ligand binding methods

For the determination of folates a ligand binding assay is very common this method is also known as competitive radio protein-binding assay (RPBA). In this method radio labeled folates in the analysis-kit compete with unlabeled folates in the sample for the binding sites of folates binding protein (FBP). The folates bounded in FBP are determined by titration with radio-labeled folates and the non-competitive assay of the sample is incubated again with FBP. The protein binding assay can be also performed by using enzyme labeled FBP (enzyme protein binding assay, EPBA), in this assay protein bound enzymatic activity is calculated by measuring the change of the substrate into product [30]. These assays are commonly used to determine folates in serum and red blood cells. However, some factors like pH of the assay medium and matrix may highly the results [31]. Thus, it is important to eliminate matrix effects during the sample extracts purification prior to analysis of folates [32]. On the other hand, the analysis of food folates is combursome process, since the affinities of folates vitamers to FBP vary [33]. Among the natural folates like H_4 folate, 5-CH_3-H_4 folate and 5-HCO-H_4 folate and two folates, PGA and (6R)-5-CH_3-H_4 folate which are not found in the nature showed affinities for bovine FBP [34]. In folates determination, ligand binding methods are also used as immunoassays which are based on the interaction of an antibody with its target molecule (antigen). Other assay in this method like enzyme-linked immunosorbent assay (ELISA) is highly specific and appropriate for folates determination in a complex matrix [35]. The radioimmunoassay (RIA) is rather limited and mainly suitable for the analysis of folic acid [36].

In general, the bio-specific assays are specific, rapid, and simple but they are often less sensitive than the microbiological assays. Several studies have proved poor correlation between ligand binding and microbiological assays because the kits of ligand binding assays given lower responses for other folate vitamers than folic acid [37].

Chromatographic assays

These methods involve in the determination of folates either folates vitamers or folates with different numbers of glutamyl residues forms. The high pressure liquid chromatography (HPLC) has been widely used in total folates analysis (Figure 2). Several HPLC methods have been also developed for determination of folate vitamers in food [18,38]. For development of HPLC methods the specific purification of the food folates is a key limiting factor. Usually, the purification procedures comprise weak or strong anion exchange, cation exchange, and affinity chromatography. Affinity chromatography is a reliable method for purification of folate samples extracts mainly from the cereals (Table 1). Most of the HPLC methods depend on either reverse phase or ion exchange separation. The ion exchange chromatography method is used in separation of polyglutamates and folate vitamers [39] and chain length of polyglutamates can be determined by cleavage of the C_9-N_{10} bond, resulting formation of para-aminobenzoylglutamates [40]. Based on basic principles, some common methods like UV, diode

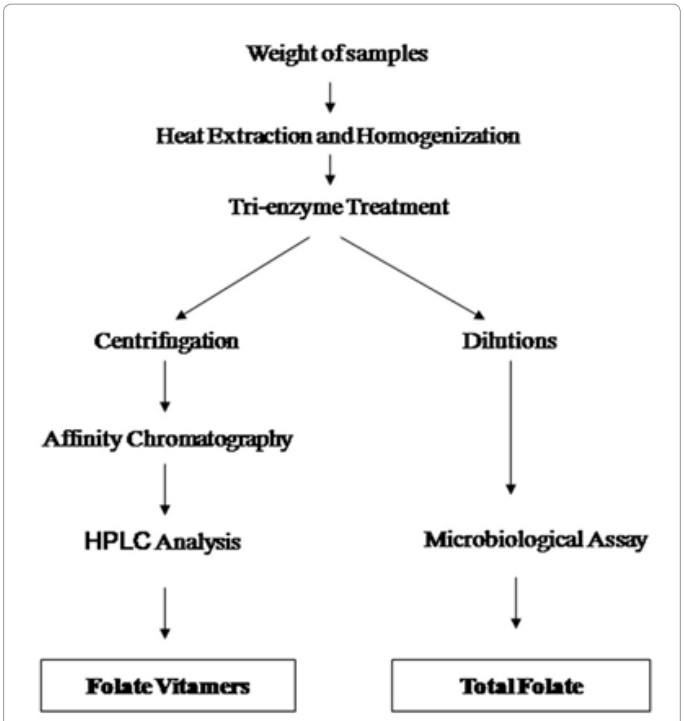

Figure 2: A systematic representation of folates analysis by HPLC method.

array, fluorescence, and electrochemical have been also used in folates determination [41]. Among the methods, fluorometric detection is accurate and more sensitive than UV detection. Recently used some common HPLC methods for folates detection are described in the table 1. The folates detection methods can be used in combinations. Detection methods also can also be used in combinations. For instance, in a sensitive, specific, quantitative HPLC method all the folate vitamers of the sample undergo a chemical pre column conversion to 5-CH_3-H_4 folate, which is then, detected fluorometrically [42]. Unfortunately, this method does not allow the determination of individual folates vitamer but offers a good alternative to the microbiological assay of total folates.

Introduction of mass spectrometric methods approach has appears very promising in folates determination; however, its applications to food folates analysis are still scarce. A LC-MS method with negative ion electro-spray has been used for detection and seperation of four folate vitamers [43]. This method has also been tested on multivitamin tablet, breakfast cereal, and beef and vegetable extract. Recently used some common HPLC methods for folates detection in different food sample extracts are summarized in the table 1.

Folates Metabolism in Plants

Enzymes of folates biosynthesis

The folates biosynthesis in plants occurs almost in similar ways as reported for microorganisms. The genetic engineering of biosynthetic pathway enzymes and related genes has been studied extensively in Arabidopsis thaliana [44]. The studies revealed that the first step comprises with synthesis of the pterin and PABA moieties in separate branches (Figure 3). PABA is synthesized in two steps from chorsimatein plastids. In the second step, enzymes GTP-cyclohydrolase I (GTPCHI; EC 3.5.4.16), dihydroneopterin triphosphate diphosphatase (DHNTPDP, EC 3.6.1.n4) and dihydroneopterin aldolase (DHNA;

Sample	Enzyme treatments	Purification	Columns	Conditions	Detection	References
Whole wheat flour	Hog kidney conjugase	DEAE-sephadex A-25	µ-Bondapak-Phenyl (300 x 3.9 mm, 10 µm)	Acetonitrile – phosphate buffer, pH 2.3, gradient	FL ex/em 295/356 nm	Gregory *et al.*[68]
Rye & wheat grains	Hog kidney conjugase	DEAE-sephadex A-25	µ-Bondapak-Phenyl and µ-Bondapak- C_{18} (300 x 3.9 mm)	Acetonitrile-phosphate buffer, pH 2.3, isocratic or gradient	FL ex/em 295/356 nm and 365/450 nm (UV 280 nm)	Müller[69]
Wheat bread, rice & pasta breakfast cereal	α-Amylase, rat plasma conjugase & protease	Affinity chromatography	PhenomenexUltremex C_{18} (250 x 4.6 mm, 5 µm)	Acetonitrile – phosphate buffer, pH 2.3, gradient	DAD 280 nm	Pfeiffer *et al.*[19]
Breads, pastries and cakes & cereal products	α -Amylase, rat plasma conjugase & protease	Affinity chromatography	Vydac 201 TP 54 (250 x 4.6 mm, 5 µm)	Acetonitrile – phosphate buffer, pH 2.1, gradient	DAD 280 nm FL ex/em 280/359 nm and 360/460 nm	Konings *et al.*[3]
Bread making flour (sponge bread)	α –Amylase, rat plasma conjugase & protease	SPE, SAX (Quaternar amine)	Microsorb- MV C_{18} (100 x 4.6, 3 µm)	Methanol – phosphate buffer pH 6.8, tetrabutyl ammoniumdihydr--ogen phosphate (ion-pairing agent), Isocratic	UV 280 nm, FL ex/em 290/350 nm or 450 nm	Osseyi *et al.*[70]
Breakfast roll	α-Amylase, Hog kidney conjugase & protease	SPE, SAX	Zorbax SB- (150 x 4.6 mm, 5 µm)	Acetonitrile – acetic acid gradient	DAD 290 nm, FL ex/em 290/360 nm	Johansson *et al.*[71]
Fortified Wheat flour	α-Amylase, rat serum conjugase & protease	SPE, SAX (quaternary amine)	Phenomenex Aqua C_{18} (250 x 4.6, 5 µm)	Acetonitrile – acetic acid gradient	stable isotope dilution assay, positive electro spray, selected reaction monitoring (SRM)	Freisleben *et al.*[72] Rychlik[73]
Whole wheat grain, triticale and rye flour, oatmeal, barley groats, rye breads	α-Amylase, rat plasma conjugase & protease	Affinity chromatography	Phenomenex Luna C_{18} (250 x 4.6, 5 µm)	Acetonitrile – phosphate buffer, pH 2.3 gradient	UV 290 nm, FL ex/em 290/356 nm and 360/460 nm	Gujska & Majewska[74]
Cereal grain products	α-Amylase, rat plasma conjugase & protease	Affinity chromatography	ODS-Hypersil (250 mm x 4.6 mm, 5 µm)	Acetonitrile-phosphate buffer	DAD 280 nm FL ex/em 295/360	Poo-Prieto *et al.*[75]
In biofortified rice	α-Amylase, deconjugase & protease	Ultra filteration (5 KDa Millipore)	Polaris C 18-A (150 mm x 4.6 mm, 3 µm)	Formic acid-water & formic acid-acetonitrile gradient	LC-MS-MS stable isotope dilution assay, positive electro spray, SRM	De Brouwer *et al.*[76]
In biofortified rice	α-Amylase, deconjugase & protease	Ultra filteration (5 KDa Millipore)	Acquity HSS T3 (150 mm x2.1 mm, 1.8 µm)	Formic acid-water & formic acid-acetonitrile gradient	UPLC-MS-MS stable isotope dilution assay, positive electro spray, SRM	De Brouwer *et al.*[77]

Table 1: HPLC methods for folate determination in foods and plants.

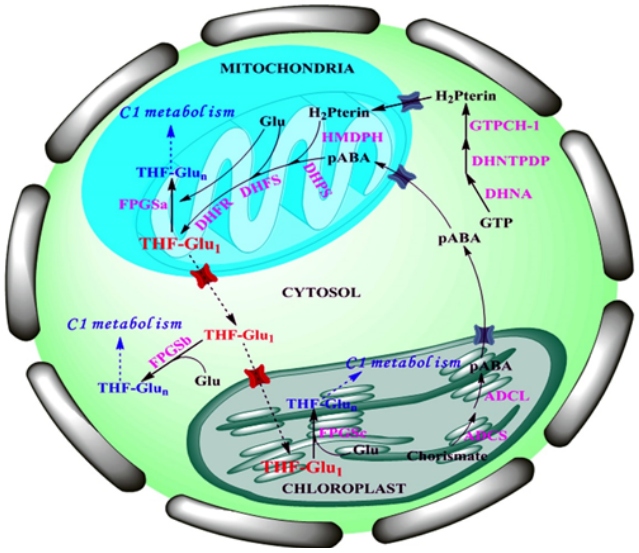

Figure 3: The tetrahydrofolate biosynthesis pathway in plants cell. The enzymes abbreviations: GTPCHI: Guanosine Triphosphate Cyclohydrolase I; DHNTPDP: Dihydroneopterin Triphosphate Diphosphatase; DHNA: Dihydroneopterin Aldolase; ADCS: Aminodeoxychorismate Synthase; ADCL: Aminodeoxychorismate Lyase; HMDHP: 6-Hydroxymethyldihydropterin Pyrophosphokinase; DHPS: Dihydropteroate Synthase; DHFS: Dihydrofolate Synthetase; DHFR: Dihydrofolate Reductase; FPGS: Folylpolyglutamate Synthase (details about each enzyme given in text).

EC 4.1.2.25) involve in the synthesis of dihydropterin from GTP. Genomic evidence suggests that these enzymes of the pterin branch are present in the cytosol. The reactions of third step occur in the stroma of the chloroplast in which enzymes aminodeoxychorismate synthase (ADCS; EC 2.6.1.85) and aminodeoxychorismate lyase (ADCL; EC 4.1.3.38) catalyze the reactions of PABA synthesis from chorismate, a product of the shikimate pathway. After synthesis of PABA in stroma it is transfered in mitochondria where dihydropterin and glutamate are present. In mitochondria, the first two reactions are catalyzed by a bifunctional 6-hydroxymethyldihydropterin pyrophosphokinase (HMDHP; EC 2.7.6.3) and dihydropteroate synthase (DHPS; EC 2.5.1.15) enzymes. In folates biosynthesis pathway, DHPS activity is feedback inhibited by dihydropteroate, a product of the reaction, dihydrofolate, and THF monoglutamate so this point is considered as a potential regulatory point. The structural analogs of PABA (sulfonamides) compounds which behave as herbicides can also inhibit DHPS activity. The attachment of the first glutamate residue to THF is catalyzed by Dihydrofolate synthase (DHFS; EC 6.3.2.12) this is first glutamylation step. For plant development, the glutamylation step is essential because a knockout in the corresponding gene can serve embryo-lethal in Arabidopsis [45]. The dihydrofolate is reduced to THF after first glutamylation step this reaction is catalyzed by dihydrofolate reductase (DHFR; EC 1.5.1.3) enzyme. Finally, DHF is reduced to THF by DHF reductase, which remains fused to thymidylate synthase in plants [46]. One residue at a time the polyglutamate tail is added in THF and its C-substituted forms by the action of folylpolyglutamate synthase (FPGS) [47]. The FPGS isoforms vary in different plant species, in Arabidopsis, three whereas in other higher plants two or more isoforms have been reported [48,49]. The fluorescent protein fusion experiment has confirmed that Arabidopsis isoforms FPGSa, FPGSb and FPGSc show to be specifically targeted to the cytosol, mitochondria, and plastids respectively. However, the experiments of single and double FPBS knockouts confirm that one or more FPBS isoforms target to multiple organelles [50]. In tomato plant, two FPGS genes are enough to target multiple orgenelles [49]. In cytosol, mitochondria, and plastids FPGS and polyglutamates occur consistently [51], this simplifies that monoglutamates are the transported forms of folate [52]. However, vacuoles are exception; they have folate polyglutamates but not contain FPGS and required ATP for further reaction [50,53].

With the advancement genetic engineering tools, significant efforts have recently been achieved to increase folates contents of plants. In an experiment, transgenic lines of GTPCHI gene from E. coli expressed in Arabidopsis showed 1000 folds more pteridine and 2-4 times more folates compared to wild plants [54]. For tissue specific expression, the mammalian GTPCHI gene was expressed in tomato fruit, as a result, the transgenic lines showed remarkable increase in pteridine content by 3 to 140-fold by an average of two fold relative to vector alone as controls [55]. Arabidopsis thaliana GTPCHI and ADCS genes also targeted for tissue specific expression in rice seeds [56,57]. The results of this experiment revealed that the ADCS transformed transgenic lines of rice alone showed 49-fold enhanced levels of PABA, in addition to around 89% of folates is present as the 5-methyltetrahydrofolate form in transgenic lines. Over-expression of A. thaliana cDNAs encoding GTPCHI and ADCS was also attempted in potato, with control of tuber-specific promoters could not enhance sufficient folates level [58]. These studies suggest that the two-gene strategies cannot be always a suitable approaches to biofortify the staple crops in order to enhance folates level [59].

Folates compartmentation and transport

The previous studies in pea leaves suggest that the plant folates are synthesized in the sub-cellular compartments i.e. cytosol, mitochondria and chloroplasts and its distribution within different sub-cellular compartment was indicated that mitochondria accounted 40%, plastids 10%, vacuoles 20% and cytosol 30% of total folates [60]. Folates synthesis is controlled by the mitochondrial photorespiration enzymes activity of serine hydroxymethyltransferase and glycine decarboxylase and the activity of these enzymes are reported more advance in green leaves than etiolated leaves [61]. The entire green leave folates concentration increases during folates synthesis whereas mitochondrial folates not enhance in similar pattern [61]. The extra folates are made exclusively in mitochondria but it arises in other compartments as well. This implies the presence of folates transporters in the mitochondrial envelope. The transport of folates from one organelle to other occurs through transporter proteins during its biosynthesis and compartmentation in the plant cells.

To date, several folates transporter proteins of mitochondria and chloroplast have been identified in plants and microorganisms. The mitochondrial petrin transporters in plants have not been identified yet however, it is believed that the role of these transporters could be analogous to that of protistan parasite Leishmania transporters. A biopetrin transpoter protein (BT1) has been well characterized which belongs to FBT1 (folates biopetrin transporter) family [62]. In Arabidopsis thaliana, two chloroplastic folates transporters AtFOLT163 [63] and At2g32040 [64] have been identified that import folates in plastids, both of these are homologs of known folates transporters. In plants, the role of AtFOLT1 has not been full characterized but the functions of this transporter well studied in Chinese hamster ovary. Another chloroplastic folates transporter At2g32040 has been expressed in E. coli it confirms role in folates transport in plants [64]. The informations about vacuolar pABA glucose ester, folate polyglutamate and folate monoglutamate transport still lacking in

their genes have also not been cloned so far [65-67]. Pterin transport in Leishmania, some studies has been conducted whereas it has not been well studied in plants.

Conclusions

It is essential to improve folates composition of foods in order to achieve better nutrition and health of the growing population. The total folates in food diets cannot be assessed without having state of the art analytical tools. The enzymes involved in folates biosynthesis in plants have been identified and well characterized. Some of the genes involved in the synthesis of these enzymes have been cloned, but we still lack the details of information related to control of flux in the pathway. The transport of folates and their precursors within and between cells is clearly a frontier for investigation. Transport systems for folates comprise of specific proteins and these need to be studied characterized in greater detail. Studies related to transport processes become difficult when carriers are present in low amounts such as for those involved in folates transport. In Arabidopsis certain transporters involved in transport of folates have been characterized that belong to different families of transporter proteins. Folates transport system within and between the cells appears to be a limiting factor for synthesis, distribution and storage and storage of folates. A detailed study of folates transport processes, characterization of transporters in higher plants will open up new possibilities to enhance folates level in plants using modern tools of genetic engineering.

References

1. Storozhenko S, Ravanel S, Zhang GF, Rebeille F, Lambert W, et al. (2005) Folate enhancement in staple crops by metabolic engineering. Trends in Food Sci and Tech 16: 271-281.
2. Cossins EA (2000) The fascinating world of folate and one carbon metabolism. Canadian J of Bot 78: 691-708.
3. Konings EJ, Roomans HH, Dorant E, Goldbohm RA, Saris Wim HM, et al. (2001) Folate intake of the Dutch population according to newly established liquid chromatography data for foods. Am J Clin Nutr 73: 765-776.
4. Morris MC, Tangney CC (2007) Is dietary intake of folate too low? Lancet 369: 166-167.
5. Krishnaswamy K, Madhavan NK (2001) Importance of folate in human nutrition. Brit J Nutr 85: 115-124.
6. Gallucci M, Zanardo A, De Valentin L, Vianello A (2004) Homocysteine in Alzheimer disease and vascular dementia. Arch Gerontl Geriatr 38: 195-200.
7. MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the medical research council vitamin study. Lancet 338: 131-137.
8. Lopez-Camelo JS, Orioli IM, Graca Dutra M, Nazer-Herrera J, Nelson R, et al. (2005) Reduction of birth prevalence rates of neural tube defects after folic acid fortification in Chile. Am J Med Gent 135: 120-125.
9. French AE, Grant R, Weitzman S, Ray JG, Vermeulen MJ, et al. (2003) Folic acid food fortification is associated with a decline in neuroblastoma. Clin Pharmacol Ther 74: 288-294.
10. Goyer A, Navarre DA (2007) Determination of folate concentrations in diverse potato germplasm using a trienzyme extraction and a microbiological assay. J Agri Food Chem 55: 3523-3528.
11. Combs GF (1992) The Vitamins-Fundamental Aspects in Nutrition and Health. Acedemic Press, San Diego, CA.
12. Blakley RL (1988) IUPAC-IUB joint commission on biochemical nomenclature (JCBN). Nomenclature and symbols for folic acid and related compounds, Recommendations 1986. J Biol Chem 263: 605-607.
13. Schirch V, Strong WB (1989) Interaction of folylpolyglutamates with enzymes in one carbon metabolism. Arch Biochem Biophys 269: 371-380.
14. Hawkes JG, Villota R (1989) Folates in foods: Reactivity, stability during processing, and nutritional implications. Crit Rev Food Sci and Nutr 28: 438-538.
15. Eitenmiller ER, Landen WO (1999) Folate. In: Vitamin Analysis for the Health and Food Sciences. CRC Press, Boca Raton, Florida.
16. Gregory JF (1989) Chemical and nutritional aspects of folate research: analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. Adv Food & Nutr Res 33: 1-101.
17. Gregory JF (1996) Vitamins. In: Food Chemistry, Fennema. Marcel Dekker, New York.
18. Maruyama T, Shiota T, Krumdieck CL (1978) The oxidative cleavage of folates. Anal Biochem 84: 277-295.
19. Pfeiffer CM, Rogers LM, Gregory JF (1997) Determination of folate in cereal-grain food products using trienzyme extraction and combined affinity and reversed-phase liquid chromatography. J Agri Food Chem 45: 407-413.
20. Puwastien P, Pinprapai N, Judprasong K, Tamura T (2005) International inter-laboratory analyses of food folate. J Food Comp Anal 18: 387-397.
21. Wilson SD, Horne DW (1984) High-performance liquid chromatographic determination of the distribution of naturally occurring folic acid derivatives in rat liver. Anal Bioch 142: 529-535.
22. Gregory JF, Engelhardt R, Bhandari SD, Sartain DB, Gustafson SK, et al. (1990) Adequacy of extraction techniques for determination of folates in foods and other biological materials. J Food Comp Anal 3: 134-144.
23. Hyun TH, Tamura T (2005) Trienzyme extraction in combination with microbiological assay in food folate analysis: an updated review. Exp Biol and Med 230: 444-454.
24. Tamura T (1998) Determination of food folate. J Nutr Bioch 9: 285-293.
25. Wilson SD, Horne DW (1982) Use of glycerol-protected Lactobacillus casei for microbiological assay of folic acid. Clin Chem 28: 1198-2000.
26. Horne DW, Patterson D (1988) Lactobacillus casei microbiological assay of folic acid derivatives in 96-well microtiter plates. Clin Chem 34: 2357-2359.
27. O'Broin S, Kelleher B (1992) Microbiological assay on microtiter plates of folate in serum and red cells. J Clin Pathol 45: 344-347.
28. Tamura T, Shin YS, Williams MA, Stokstad ELR (1972) Lactobacillus casei response to pteroylpolyglutamates. Anal Biochem 49: 517-521.
29. Goli DM, Vanderslice JT (1989) Microbiological assay of folacin using a CO2 analyzer system. J Micronutr Anal 6: 19-23.
30. Finglas PM, Faulks RM, Morgan MRA (1988) The development and characterization of a protein-binding assay for the determination of folate-potential use in food analysis. J Micronutr Anal 4: 295-308.
31. Van H, Finglas PM, Bates C (1994) FLAIR intercomparisons on serum and red cell folate. Intern J Vit and Nutr Res 64: 288-293.
32. Strålsjö L, Åhlin H, Witthöft CM, Jastrebova J (2003) Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high performance liquid chromatography (HPLC). Eur Food Res Technol 216: 264-269.
33. Strålsjö L, Arkbåge K, Witthöft C, Jägerstad M (2002) Evaluation of a radio protein binding Assay (RPBA) for folate analysis in berries and milk. Food Chem 79: 525-534.
34. Nygren-Babol L, Sternesjö Å, Jägerstad M, Björck L, et al. (2005) Affinity and rate constants for interactions of bovine folate-binding protein and folate derivatives determined by optical biosensor technology. Effect of stereoselectivity. J Agri and Food Chem 53: 5473-5478.
35. Finglas PM, Morgan MRA (1994) Application of biospecific methods to the determination of B-group vitamins in food-a review. Food Chem 49: 191-201.
36. Keagy PM (1985) Folacin. Microbiological and animal assays. Wiley & Sons, New York.
37. Finglas PM, Faure U, Southgate DAT (1993) First BCR-intercomparison on the determination of folates in food. Food Chem 46: 199-213.
38. Konings EJM (1999) A validated liquid chromatographic method for determining folates in vegetables, milk powder, liver, and flour. J AOAC Intern 82: 119-127.
39. Seyoum E, Selhub J (1993) Combined affinity and ion-pair column chromatographies for the analysis of food folate. J Nutr Biochem 4: 488-494.

40. Eto I, Krumdieck CL (1982) Determination of three different pools of reduced one-carbonsubstituted folates. III. Reversed-phase high performance liquid chromatography of the azo dye derivatives of p-aminobenzoylpoly-- glutamates and its application to the study of unlabeled endogenous pteroylpolyglutamates of rat liver. Anal Biochem 120: 323-329.

41. Bagley P, Selhub J (2000) Analysis of folate form distribution by affinity followed by reversed-phase chromatography with electrochemical detection. Clin Chem 46: 404-411.

42. Ndaw S, Bergaentzlé M, Aoudé-Werner D, Lahély S, Hasselmann C, et al. (2001) Determination of folates in foods by high-performance liquid chromatography with fluorescence detection after precolumn conversion to 5-methyltetrahydrofolates. J Chromatogr A 928: 77-90.

43. Stokes P, Webb K (1999) Analysis of some folate monoglutamates by high-performance liquid chromatography-mass spectrometry. J Chromatogr A 864: 59-67.

44. Rébeillé F, Ravanel S, Jabrin S, Douce R, Storozhenko S, et al. (2006) Folates in plants: biosynthesis, distribution, and enhancement. Physiol Plant 126: 330-342.

45. Ishikawa T, Machida C, Yoshioka Y, Kitano H, Machida Y, et al. (2003) The GLOBULAR ARREST1 gene, which is involved in the biosynthesis of folates, is essential for embryogenesis in Arabidopsis thaliana. Plant J 33: 235-244.

46. Cox K, Robertson D, Fites R (1999) Mapping and expression of a bifunctional thymidylate synthase, dihydrofolate reductase gene from maize. Plant Mol Biol 41: 733-739.

47. Ravanel S, Cherest H, Jabrin S, Grunwald D, Surdin-Kerjan Y, et al. (2001) Tetrahydrofolate biosynthesis in plants: molecular and functional characterization of dihydrofolate synthetase and three isoforms of folylpolyglutamate synthetase in Arabidopsis thaliana. Proc Nat Acad Sci USA 98: 15360-15365.

48. McIntosh SR, Henry RJ ((2008) Genes of folate biosynthesis in wheat. J Cereal Sci 48: 632-638.

49. Waller JC, Akhtar TA, Lara-Nunez A, Gregory JF, McQuinn RP, et al. (2010) Developmental and feed forward control of the expression of folate biosynthesis genes in tomato fruit. Mol Plant 3: 66-77.

50. Mehrshahi P, Gonzalez-Jorge S, Akhtar TA, Ward JL, Santoyo-Castelazo A, et al. (2010) Functional analysis of folate polyglutamylation and its essential role in plant metabolism and development. Plant J 64: 267-279.

51. Orsomando G, Diaz GR, Green BJ, Peng M, Rea PA, et al. (2005) Plant γ-glutamyl hydrolases and folate polyglutamates: characterization, compartmentation and co-occurrence in vacuoles. J Biol Chem 280: 28877-28884.

52. Suh JR, Herbig AK, Stover PJ (2001) New perspectives on folate catabolism. Ann Rev Nutr 21: 255-282.

53. Farre EM, Tiessen A, Roessner U, Geigenberger P, Trethewey RN, et al. (2001) Analysis of the compartmentation of glycolytic intermediates, nucleotides, sugars, organic acids, amino acids, and sugar alcohols in potato tubers using a nonaqueous fractionation method. Plant Physiol 127: 685-700.

54. Hossain T, Rosenberg I, Selhub J, Kishore G, Beachy R, et al. (2004) Enhancement of folates in plants through metabolic engineering. Proc Nat Acad Sci USA 101: 5158-5163.

55. Diaz GR, Gregory JF, Hanson AD (2007) Folate biofortification of tomato fruit. Proc Nat Acad Sci USA 104: 4218-4222.

56. Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, et al. (2007) Folate fortification of rice by metabolic engineering. Nature Biotechnol 25: 1277-1279.

57. Blancquaert D, Van DJ, Storozhenko S, Stove C, Lambert W, et al. (2013) Rice folate enhancement through metabolic engineering impacts rice seed metabolism, but does not affect the expression of the endogenous folate biosynthesis genes. Plant Mol Biol 83: 329-349.

58. Blancquaert D, Storozhenko S, Van DJ, Stove C, Visser R, et al. (2013) Enhancing pterin and paraaminobenzoate content is not sufficient to successfully biofortify potato tubers and Arabidopsis thaliana plants with folate. J Exp Bot 64: 3899-3909.

59. De Steur H, Gellynck X, Blancquaert D, Lambert W, Van Der Straeten D, et al. (2012) Potential impact and cost-effectiveness of multi-biofortified rice in China. New Biotechnol 29: 432-442.

60. Jabrin S, Ravanel S, Gambonnet B, Douce R, Rebeille F, et al. (2003) One-carbon metabolism in plants. Regulation of tetrahydrofolate synthesis during germination and seedling development. Plant Physiol 131: 1431-1439.

61. Gambonnet B, Jabrin S, Ravanel S, Karan M, Douce R, et al. (2001) Folate distribution during higher plant development. J Sci Food and Agri 81: 835-841.

62. Lemley C, Yan S, Dole VS, Madhubala R, Cunningham ML, et al. (1999) The Leishmania donovani LD1 locus gene ORFG encodes a biopterin transporter (BT1). Mol and Biochem Parasitol 104: 93-105.

63. Bedhomme M, Hoffmann M, McCarthy EA, Gambonnet B, Moran RG, et al. (2005) Folate metabolism in plants: An Arabidopsis homolog of the mammalian mitochondrial folate transporter mediates folate import into chloroplasts. J Biol Chem 280: 34823-34831.

64. Klaus SM, Kunji ER, Bozzo GG, Noiriel A, Diaz GR, et al. (2005) Higher plant plastids and cyanobacteria have folate carriers related to those of trypanosomatids. J Biol Chem 280: 38457-38463.

65. Dean JV, Mills JD (2004) Uptake of salicylic acid 2-O-β-D-glucose into soybean tonoplast vesicles by an ATP-binding cassette transporter-type mechanism. Physiol Plant 120: 603-612.

66. Klaus SM, Wegkamp A, Sybesma W, Hugenholtz J, Gregory JF, et al. (2005) A nudix enzyme removes pyrophosphate from dihydroneopterin triphosphate in the folate synthesis pathway of bacteria and plants. J Biol Chem 280: 5274-5280.

67. Eudes A, Kunji ER, Noiriel A, Klaus SM, Vickers TJ, et al. (2010) Identification of transport-critical residues in a folate transporter from the folate-biopterin transporter (FBT) family. J Biol Chem 285: 2867-2875.

68. Gregory JF, Sartain DB, Day BPF (1984) Fluorometric determination of folacin in biological materials using high performance liquid chromatography. J Nutr 114: 341-353.

69. Müller H (1993) Bestimmung der Folsäure-Gehalte von Getreide, Getreideprodukten, Backwaren und Hülsenfrüchten mit Hilfe der Hochleistungsflüssigchromatographie (HPLC). Forsch Komplementarmed 197: 573-577.

70. Osseyi ES, Wehling RL, Albrecht JA (2001) HPLC determination of stability and distribution of added folic acid and some endogenous folates during breadmaking. Cereal Chem 78: 375-378.

71. Johansson M, Witthöft C, Bruce Å, Jägerstad M (2002) Study of wheat breakfast rolls fortified with folic acid. The effect on folate status in healthy women during a 3-month intervention. Eur J Nutr 41: 279-286.

72. Freisleben A, Schieberle P, Rychlik M (2003) Specific and sensitive quantification of folate vitamers in foods by stable isotope dilution assays using high-performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 376: 149-156.

73. Rychlik M (2004) Revised folate content of foods determined by stable isotope dilution assays. J Food Comp Anal 17: 475-483.

74. Gujska E, Majewska K (2005) Effect of baking process on added folic acid and endogenous folates stability in wheat and rye breads. Plant Food Hum. J Nutr 60: 37-42.

75. Poo-Prieto R, Haytowitz DB, Holden JM, Rogers G, Choumenkovitch SF, et al. (2006) Use of the affinity/HPLC method for quantitative estimation of folic acid in enriched cereal-grain product. J Nutr 136: 3079-3083.

76. Brouwer DV, Storozhenko S, Van DJ, Wille SRM, Stove CP, et al. (2008) Optimisation and Validation of liquid chromatography-tandem mass spectrometry method for folate in rice. J Chromatogr A 1215: 125-132.

77. Brouwer DV, Storozhenko S, Stove CP, Van Daele J, Van DS, et al. (2010) Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) for the sensitive determination of folate in rice. J Chromatogr B 878: 509-513.