LWT - Food Science and Technology 63 (2015) 599–603

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Mechanism of folic acid radiolysis in aqueous solution

Michel M. Araújo [a, b, c, *], Eric Marchioni [b, c], Anna Lucia C.H. Villavicencio [a], Minjie Zhao [b, c], Thomas di Pascoli [b, c], Florentz Kuntz [d], Martine Bergaentzle [b, c]

[a] Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP), Centro de Tecnologia das Radiações, Av. Prof. Lineu Prestes 2242, 05508-910 São Paulo, Brazil
[b] Université de Strasbourg, IPHC, 74, route du Rhin, 67400 Illkirch, France
[c] CNRS, UMR7178, 67400 Illkirch, France
[d] Aérial, Institut Technique Agro-Industriel, Parc d'Innovation, 250 rue Laurent Fries, B.P. 40443, F-67412 Illkirch, France

ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form
8 March 2015
Accepted 11 March 2015
Available online 19 March 2015

Keywords:
Folic acid
Vitamin
Degradation products
E-beam
Irradiation

Chemical compounds studied in this article:
Folic acid (PubChem CID: 6037)
4aminobenzoic acid (PubChem CID: 978)
p-aminobenzoyl-L-glutamic acid (PubChem CID: 196473)
N-(4-nitrobenzoyl)-L-glutamic acid hemihydrate (PubChem CID: 95525)
pterine-6-carboxylic acid (PubChem CID: 70361)
pteroic acid (PubChem CID: 95054)
xanthopterin monohydrate (PubChem CID: 2723796)
6-(hydroxymethyl)pterin (PubChem CID: 69736)

ABSTRACT

Folates compounds are a B group vitamin vital for important biochemical processes like DNA synthesis and repair and in certain biological reactions as a cofactor. Folic acid (FA) is composed of a pteridine ring, *p*-aminobenzoic acid and glutamate moieties. Separately, the three moieties have no vitamin activity. Folate deficiency can lead to increased risk of several pathologies. FA is known to be a sensitive compound, easily degraded by pH, light, heat and food processing. Food irradiation is a process exposing food to ionizing radiations to reduce storage losses, extend shelf life and microbiological safety. Radiation treatment produces oxygen radicals and thereby induces oxidative damage in biomolecules such as proteins, lipids, DNA and vitamins. In the present work, aqueous FA solutions are submitted to electron-beam (E-beam) radiation in a dose range of 0.25–10 kGy. Upon irradiation, main FA radio-products are quantified by HPLC. E-beam processing undergoes radiolysis to yield some known FA photoproducts and also new radio-products are formed: 6-(hydroxymethyl)pterin and N-(4-nitrobenzoyl)-L-glutamic acid. A radio-degradation pathway of FA is also discussed.

1. Introduction

Folate compounds are a B group vitamin, vital for important biochemical processes like DNA synthesis and repair and in certain biological reactions as a cofactor (Lucock, 2000).

Folate deficiency can induce diseases such as megaloblastic anemia, neural tube defects, cardiovascular diseases, cancer and has also been associated with age-related cognitive decline, particularly in older adults (Durga et al., 2007; Moat et al., 2004; Rucker, Suttie, McCormick, & Machlin, 2001). FA is the synthetic form of folate and is found in food supplements and fortified food. FA is used for this purpose because it is cheaper and more stable than physiological types of folate (McNulty & Pentieva, 2004). Folic acid (FA) or pteroylglutamic acid is composed of a pteridine ring, *p*-aminobenzoic acid (PABA) and glutamate moieties. Separately, the three moieties have no vitamin activity (Lucock, 2000; USP, 1975).

Food treatments intend the preservation of nutritional quality and microbiological safety during food processing. The best compromise between the goal of the food treatment and the

* Corresponding author. Université de Strasbourg, IPHC, 74, route du Rhin, 67400 Illkirch, France. Tel.: +33 368854159; fax: +33 368854392.
E-mail address: mmozeika@yahoo.com (M.M. Araújo).

http://dx.doi.org/10.1016/j.lwt.2015.03.038
0023-6438/

preservation of nutritional quality involves optimizing the intensity of treatment and knowledge of the transformation process. Radiation treatment is a process exposing food to ionizing radiations such as gamma rays emitted from the radioisotopes ^{60}Co or high energy electrons and X-rays produced by machine sources (Farkas, 2006). Irradiation produces oxygen radicals and thereby induces oxidative damage in biomolecules such as proteins, lipids and DNA (Riley, 1994). FA is known to be a sensitive compound, easily degraded by pH, light, heat and food processing (Ball, 2006; Delchier et al., 2014; Hefni & Witthöft, 2014).

In recent years, the agrifood sector, due to the globalization and the development of new technologies, is undergoing radical changes that require a deeper characterization of the food chain, starting from raw materials up to the final products. In addition, the consumers are concerned about the food they eat and ask for more assurances on the quality, the safety and the geographical origin of the products that they consume (Di Stefano et al., 2012). Despite few reports on FA radio-degradation found in literature (Araújo et al., 2011; El-Dessouky, Abd-Elwahab, & Turk, 1988; Kesavan, Pote, Batra, Viswanathan, 2003; Nakken & Pihl, 1966) much is still let to be known, specially on the mechanism of FA radiolysis. The purpose of the present work is to identify and quantify by HPLC the radio-degradation products of folic acid aqueous solutions submitted to increasing doses of ionizing radiation and to investigate the possible radio-degradation mechanism of this vitamin.

2. Materials and methods

2.1. Chemicals

Methanol (MeOH) (Carlo Erba, Val de Reuil, France) were HPLC grade. All other chemicals were of analytical grade. Acetic acid was purchased from Riedel-de Haen (Seelze, Germany). Sodium hydroxide (NaOH) was purchased from SDS (Peypin, France). FA was obtained from Sigma–Aldrich (Steinheim, Germany). FA degradation products, xanthopterin monohydrate (XA) was purchased from Acros Organics (Geel, Belgium), 6-(hydroxymethyl)pterin (AHMP) was obtained from Florida Center for Heterocyclic Compounds (Florida, USA) and pterine-6-carboxylic acid (PCA), *p*-aminobenzoyl-L-glutamic acid (pABGA), 4-aminobenzoic acid (PABA), N-(4-nitrobenzoyl)-L-glutamic acid hemihydrate (pNBGA), pteroic acid (PA) were obtained from Sigma–Aldrich (Steinheim, Germany). Water was purified using a Synergy Milli-Q System (Millipore, Molsheim, France).

2.2. Samples

All FA aqueous solutions were prepared under subdued light, to protect folates from oxidative degradation induced by light. A total of 100 mg of FA was added to 100 mL of water and solubilized by the addition of 5.0 mL of 1 mol/L NaOH. The solution was diluted in water to a final concentration of 100 μg/mL (pH 8.3). All samples (4 mm thick) were packed in polyamide/polyethylene plastic sachets, sealed and labeled with their respective radiation doses. PABA, pABGA and pNBGA were dissolved in water. PCA, PA, XA and AHMP were dissolved in water with addition of NaOH 1 mol/L until solubilization. All degradation products standards solutions were diluted in water at a final concentration of 100 μg/mL.

2.3. Irradiation treatment

FA aqueous solutions were irradiated in five replicates in a Van de Graaff E-beam accelerator, 2 MeV (Vivirad High Voltage, Handschuheim, France) with a 100 μA current, 20 cm scan width, and about 2 kGy/s dose rate. Applied doses were around 0.25, 0.50, 0.75, 1.0, 3.0, 5.0, 7.0, 10.0 kGy and 0 (control). Surface absorbed doses were monitored with FWT 60.00 radiochromic dosimeters (Far West Technology, Goleta, CA), previously calibrated with an alanine dosimeter (Aérial, Illkirch, France) (Kuntz, Pabst, Delpech, Wagner, & Marchioni, 1996). Dose uniformity of about 10% within the sample was achieved by the use of a 100 μm thick copper scattering foil (Kuntz, Marchioni, & Strasser, 1991). Standard conditions for temperature and pressure were used in irradiation plant (25 °C and 1 atm).

2.4. HPLC-MS

Experimental work was performed using a Varian ProStar HPLC system (Palo Alto, CA) [210 binary HPLC pump, 410 AutoSampler and 335 diode array detection (DAD) UV–Vis detector] and a Varian 1200L triple quadrupole mass spectrometer system (MS) (Varian, LesUlis, France). LC/UV and LC/MS were used respectively for quantification and identification purposes. High-purity nitrogen (Domnik Hunter, Villefranche sur Saône, France) was used as nebulizing gas (344.73 kPa) and drying gas (200 °C). Needle and shield voltage were 5000 V and 600 V, respectively. The spectrometer was operated in full scan mode (70–500 m/z) using positive electrospray ionization (ESI+). Chromatographic separation was carried out on an Agilent-XDB Phenyl analytical column (250 mm × 4.6 mm; 5 μm particle size) and the eluate was split 1:5 to MS detector and 4:5 to DAD detector. Mobile phase consisted of aqueous acetic acid (0.5%) and MeOH, at a flow rate of 1.0 mL/min. Gradient elution started at 100% aqueous acetic acid for 10 min, followed by raising the MeOH concentration linearly to 45% within 42 min. Subsequently, MeOH concentration decreased to 0% in 1 min and then the column was equilibrated at this condition for 12 min. The injection volume was 20 μL. Detection was performed at 280 nm. A negative (H_2O) and a positive control (100 μg/mL FA aqueous solution) were injected throughout the sample analysis every 10 injections to control the analytical system.

2.5. Quantification of FA and FA degradation products

Prior to quantification, FA aqueous solutions (irradiated and non-irradiated) were pooled together and concentrated 10 times at 40 °C using a SpeedVac concentrator Savant SPD 121P (Thermoscientific, EUA) in order to allow the quantification of the less generated radio-products. Quantification of FA, XA, pABGA, pNBGA, AHMP and PCA was performed by external calibration by plotting peak areas versus compounds concentrations. Linear calibration curves were obtained from 1.6 to 100.0 μg/mL using at least six calibrators for each compound. Limit of detection (LOD) and limit of quantification (LOQ) values determination were based on the standard deviation of residuals according to ICH Q2B (1996). Briefly, they were calculated as the ratio between the standard deviation of residuals and the slope of calibration curve. The obtained value was multiplied by 3.3 or 10, respectively to LOD and LOQ.

3. Results and discussion

E-beam processing degraded intensively FA molecule (Fig. 1A–D). Degradation of FA molecule was followed by the formation of several degradation products (Fig. 1). Increasing radiation doses enhanced FA breakdown up to 10 kGy. It can be assumed that with radiation doses up to 1 kGy, mainly products with low-polarity were formed, nearby FA retention time (42.4 min). Radiation doses over 1 kGy degraded intensely FA molecule into more polar fragments. These results are in accordance with others