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Protection of resveratrol against the photodecomposition of folic acid and photodecomposition-induced structural change of beta-lactoglobulin

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6-Formylpterin (PubChem CID: 150847)
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ABSTRACT

Folic acid is a synthetic form of the B-group vitamin known as folate and essential for a variety of physiological processes. However, it decomposes under UV irradiation, causing indirect oxidation and structural change of protein. In this study, the protective effect of resveratrol against the photodecomposition of folic acid and its caused protein structural change was investigated by using fluorescence and absorbance spectroscopy, high performance liquid chromatography and ABTS assay. It was found that resveratrol could inhibit the folic acid decomposition and control the decomposition process, depending on the polyphenol concentration and addition time. Transformation of resveratrol was accelerated by photodecomposition of folic acid. Antioxidant activity of resveratrol was important for the protective effect. Moreover, resveratrol could also inhibit the unfolding of beta-lactoglobulin caused by the folic acid decomposition by using circular dichroism.

1. Introduction

The B-group vitamins, including thiamine, riboflavin, niacin, pantothenic acid, pyridoxal, biotin, folate and cyanocobalamin, are water-soluble and normally present in cereals, vegetables and many other foods (Capozzi, Russo, Dueñas, López, & Spano, 2012). These vitamins are required as cofactors for enzymes essential in cell function and energy production (Kennedy, 2016). Folate functions as a coenzyme in one-carbon transfer reactions required in the biosynthesis of nucleic acids and proteins (Butzbach & Epe, 2013; FAO/WHO, 2005). The B-group vitamins have to be obtained from the diet since they are not synthesized de novo by human body (Capozzi et al., 2012). However, they are usually sensitive to many environmental factors during food processing and storage, causing micronutrient deficiency and even many kinds of diseases (Capozzi et al., 2012; Cohn, 2002; Frommherz et al., 2014 and Thomas, Kumar, Sharma, Issarani, & Nagori, 2008). Folate deficiency may lead to neural tube defects in infants, vascular diseases and some cancers (Lucock, 2000; Ohrvik & Witthoft, 2011). Micronutrient deficiency may be resolved through the use of supplements and fortification strategies on the basis of the protection of the B-group vitamins by using encapsulation technology or antioxidant addition (Joshi, Gray, & Keane, 2012; Lee, Jung, & Kim, 1998; Tofzikovskaya, O'Connor, & McNamara, 2012 and Trang, Kurogi, Katsuno, Shimamura, & Ukeda, 2008).

Folate is a generic term for a naturally occurring family of B-group vitamins comprising an aromatic pteridine ring linked to *p*-aminobenzoic acid and one or more glutamate residues. Folic acid (FA, Fig. 1) is the most oxidized, stable, easily absorbable and synthetic form of folate (Dántola et al., 2010). Folate and folic acid are reduced in the intestinal mucosal cells to form bioactive tetrahydro forms (FAO/WHO, 2005). However, folate and folic acid are sensitive to ultraviolet (UV) light. Photodecomposition of folate influences the evolution of human

Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt); β-LG, beta-lactoglobulin; CD, circular dichroism; FA, folic acid; FPT, 6-formylpterin; HBD, *p*-hydroxybenzaldehyde; HPLC, high performance liquid chromatography; $K_2S_2O_8$, potassium persulfate; $^1O_2$, singlet oxygen; PCA, 6-carboxypterin; PGA, *p*-aminobenzoylglutamate; UV, ultraviolet

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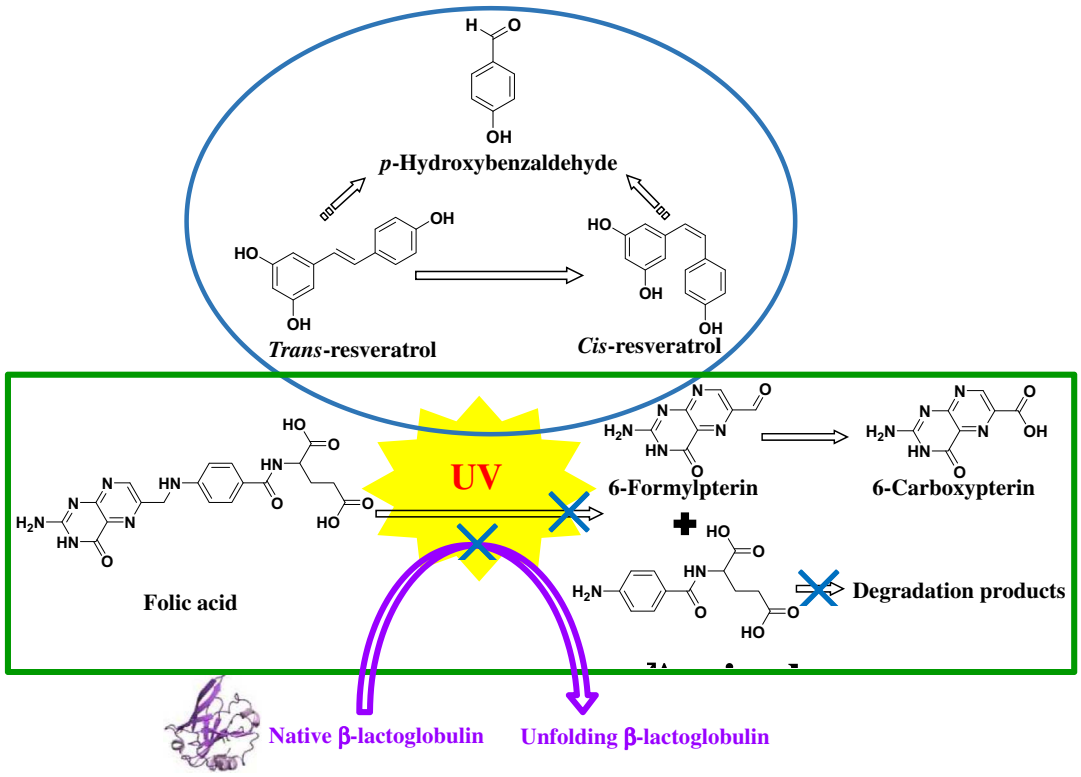

Fig. 1. Photo-induced transformation of folic acid and resveratrol and scheme of resveratrol inhibition against the decomposition of folic acid and resulting structural change of β-lactoglobulin under UV radiation, × denotes inhibition.

skin color, since the concentration of melanin increases to provide a protective effect against folate photolysis (Cohn, 2002; Jablonski & Chaplin, 2000). Folate is photogenotoxic in mammalian cells, leading to the oxidation of DNA under irradiation (Butzbach & Epe, 2013). Interaction with photodecomposition products of folic acid causes unfolding or decomposition of globular proteins (Liang, Zhang, Zhou, & Subirade, 2013).

When exposed to UV light, folic acid degrades by breakage of the C9-N10 bond to yield inactive 6-formylpterin (FPT, Fig. 1) and *p*-aminobenzoylglutamate (PGA, Fig. 1), followed by photo-oxidation of FPT to form 6-carboxypterin (PCA, Fig. 1) and photodecomposition of PCA to form non-pteridinic products and by photodecomposition of PGA to form *p*-aminobenzoic acid and glutamic acid (Akhtar, Khan, & Ahmad, 1999; FAO/WHO, 2005; Gazzali et al., 2016; Off et al., 2005 and Thomas, Suárez, Cabrerizo, Martino, & Capparelli, 2000). Pterin and its derivatives are photochemically reactive in aqueous solution and could undergo photooxidation to generate reactive oxygen species, and to photosensitize the oxidation of biomolecules mainly through an electron transfer-mediated process (Castaño, Dántola, Oliveros, Thomas, & Lorente, 2013; Dántola, Zurbano, & Thomas, 2015; Thomas et al., 2013 and Thomas et al., 2014).

The photodecomposition of folic acid could be delayed or inhibited by interacting with many proteins including serum albumins, whey proteins and caseins to form complexes (Bourassa, Hasni, & Tajmir-Riahi, 2011; Liang et al., 2013; Liang & Subirade, 2010; Vorobey, Steindal, Off, Vorobey, & Moan, 2006 and Zhang et al., 2014). Beta-lactoglobulin (β-LG, Fig. 1), the most abundant protein in bovine milk whey, is a small globular protein folded into a calyx formed by eight antiparallel β-strands and an α-helix located at the outer surface of the β-barrel. β-LG contains multiple ligand-binding sites and could bind a wide range of low-molecular weight compounds (Kanakis, Tarantilis, Polissiou, & Tajmir-Riahi, 2013; Le Maux, Bouhallab, Giblin, Brodkorb, & Croguennec, 2014; Liang et al., 2013; Liang & Subirade, 2010, 2012 and Liang, Tajmir-Riahi, & Subirade, 2008). Folic acid is possibly bound at the surface hydrophobic pocket in a groove between the α-helix and the β-barrel of β-LG, with a binding constant of $4.3 \times 10^5\ M^{-1}$ (Liang & Subirade, 2010). Moreover, folic acid and resveratrol (*trans*-3,4′,5-trihydroxystilbene, Fig. 1) could simultaneously interact with β-LG to form complexes (Zhang, Liu, Subirade, Zhou, & Liang, 2014), since resveratrol is bound to β-LG with the binding constant between 10^4 and $10^6\ M^{-1}$ and the binding site possibly at the outer surface near Trp19-Arg124 (Liang et al., 2008; Liang & Subirade, 2012). In the β-LG complexes, the photodecomposition of folic acid could be inhibited by resveratrol (Zhang et al., 2014).

Addition of antioxidants, such as ascorbic acid or aminoreductone, could reportedly provide protective effect against the photodecomposition of riboflavin (Lee et al., 1998; Trang et al., 2008). *Trans*-resveratrol, a natural polyphenol, possesses antioxidant, anti-inflammatory, anti-angiogenic and neuroprotective properties (Bastianetto, Ménard, & Quirion, 2015; Lançon, Frazzi, & Latruffe, 2016). In this study, photo-induced changes of resveratrol and/or folic acid were systematically investigated by characterization of both and their photodecomposition products, to discuss protective mechanism of resveratrol on loss of folic acid. Moreover, the protection of resveratrol against folic acid decomposition induced-structural change of β-LG was also investigated. The data gathered here should be useful for the protection of B-group vitamins and for the fortification of β-LG-contained products with B-group vitamins.

2. Materials and methods

2.1. Materials

Folic acid (~98%), 6-carboxypterin (> 98%), resveratrol (*trans*-isomer, > 99%) and β-LG (B variant, > 90%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 6-Formylpterin was purchased from Schircks Laboratories (Jona, Switzerland). *p*-Aminobenzoylglutamate, *p*-hydroxybenzaldehyde (HBD) and ABTS