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Nonenzymatic glycation reaction of folate with reducing sugars: A case study on [6S]-5-methyltetrahydrofolate and fructose

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Abstract

In the Maillard reaction, free amino groups react with reducing sugars to form nonenzymatic glycation products. It has recently been shown that in the presence of reducing sugars (i.e. fructose) folic acid is subjected to a non-enzymatic glycation reaction, which may represent an important pathway of folate degradation besides the established oxidative degradation pathways. In the current study, the thermal stability of [6S]-5-methyltetrahydrofolic acid ([6S]-5-CH_3H_4PteGlu, 0.4µM), the predominant naturally occuring folate derivate, was investigated on a qualitative and kinetic basis in the presence of different fructose concentrations (0-3M) in milliQ water (8.11ppm O_2). Samples were isothermally treated and subsequently residual concentrations of [6S]-5-CH_3H_4PteGlu were determined with RP-HPLC using fluorescence and UV-VIS detection with external calibration curves. Kinetic parameters were estimated using linear regression analysis. Addition of fructose (44µM - 3M) prior to thermal treatment (40-100°C, 15 min) resulted in destabilisation of [6S]-5-CH_3H_4Pteglu depending on the fructose concentration and the severity of the treatment. Thermal degradation kinetics (40-90°C of [6S]-5-CH_3H_4PteGlu with or without addition of fructose (1.5M) could be described by first order reaction kinetics. Addition of fructose (1.5M) prior to thermal treatments influenced (i) the estimated degradation rate constants, and influenced (ii) the estimated temperature dependence of the rate constants, resulting in a higher [6S]-5-CH_3H_4PteGlu instability. MS analysis of a heated (90°C, 120min) [6S]-5-CH_3H_4PteGlu solution (0.4µM [6S]-5-CH_3H_4PteGlu, 1.5M fructose) showed a folate degradation product with m/e 532, suggesting that natural folates are probably subjected to a similar non-enzymatic glycation reaction with fructose as has previously been shown for folic acid.

Keywords: folate, temperature, stability

Introduction

Folate is the generic term for folic acid (PteGlu) and related compounds exhibiting the same biological activity. Folates attract considerable interest for their essential role in human health and the prevention of several health disorders. Food folates occur in nature as reduced forms of folic acid and consist of many derivatives. In fruit and vegetables, [6S]-5-methyltetrahydrofolate ([6S]-5-CH_3PteH_4Glu) is the predominant natural folate form, whereas PteGlu does not naturally occur in foods but is used for fortification. It is well established that [6S]-5-CH_3PteH_4Glu is unstable during thermal processing (Nguyen et al., 2003, Indrawati et al., 2004) and that addition of

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antioxidants results in a stabilization of the natural vitamin (Indrawati et al., 2004, Oey et al., 2006). It has previously been shown that in the presence of reducing sugars (i.e. fructose) PteGlu is subjected to a non-enzymatic glycation reaction, which may represent an important pathway of folate degradation besides the known oxidative degradation (Schneider et al., 2002, Rychlik and Mayr, 2005).

Objective

The goals of the current study were i) to investigate the stability of [6S]-5-CH_3PteH_4Glu in the presence of a reducing sugar (i.e. fructose) on a kinetic basis and ii) to identify possible reaction products.

Methods

Samples and treatments: Stability of [6S]-5-CH_3PteH_4Glu (0.4 µM) with addition of fructose (0 - 3M) was investigated in milliQ water (8.11 ppm O_2). Samples were isothermally treated in a waterbath (40 - 90°C) for 30 min and subsequently cooled and stored in ice water to stop the treatment before analysis. Kinetic experiments (40 – 90°C) were conducted with samples of 0.4 µM [6S]-5-CH_3PteH_4Glu in the presence of 1.5 M fructose. Samples were treated under isothermal conditions (40 – 90°C) and the treatment was stopped at predetermined time intervals before analysis of residual of [6S]-5-CH_3PteH_4Glu concentrations. Kinetic parameters were estimated using linear regression analysis.

Folate analysis: Residual and initial [6S]-5-CH_3PteH_4Glu concentrations were determined with RP-HPLC using fluorescence (λ_{ex} 280 nm; λ_{em} 359 nm) and UV-VIS (λ 290 nm) detection with external calibration. For identification of folate degradation products, a thermally treated sample (90°C, 120 min) of [6S]-5-CH_3PteH_4Glu with 1.5 M fructose was injected directly into a mass spectrometer operated in positive electrospray ionization mode.

Results

Addition of fructose (44 µM – 3 M) prior to thermal treatment (40 – 90°C, 30 min) resulted in a destabilization of [6S]-5-CH_3PteH_4Glu depending on the fructose concentration and the severity of the treatment. Thermal degradation kinetics (40 - 90°C) of [6S]-5-CH_3PteH_4Glu with or without addition of fructose could be described by first order reaction kinetics. Addition of fructose (1.5 M) prior to the treatments resulted in higher degradation rate constants for [6S]-5-CH_3PteH_4Glu in comparison to the folate degradation rate in absence of fructose. Secondly fructose (1.5 M) influenced the estimated temperature dependence of the rate constants, showing a lower temperature dependence when fructose was added to the samples prior to the treatment. MS analysis of a heated (90°C, 120 min) [6S]-5-CH_3PteH_4Glu solution (0.4 µM) with fructose (1.5M) suggested that natural folates are probably subjected to a similar nonenzymatic glycation reaction with fructose, as has been reported for PteGlu (Schneider et al., 2002).

Conclusion

Addition of fructose to [6S]-5-CH_3PteH_4Glu solutions prior to thermal treatments, resulted in a higher folate instability, probably caused by a nonenzymatic glycation reaction.

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