Food Research International 63 (2014) 456–463

Contents lists available at ScienceDirect

Food Research International

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

In vitro absorption studies of chlorogenic acids from coffee using the Ussing chamber model

Denise Scherbl, Sabrina Muentnich, Elke Richling *

Division of Food Chemistry and Toxicology, Department of Chemistry, University of Kaiserslautern, Germany

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 19 February 2014
Accepted 27 March 2014
Available online 5 April 2014

Keywords:
Coffee
Chlorogenic acids
Ussing chamber
Caffeoylquinic acid lactones
Caco-2 cells
Hydroxycinnamic acids

ABSTRACT

Hydroxycinnamates are secondary plant metabolites and ubiquitous constituents of plant materials, including many foods and beverages. Hydroxycinnamates are most often conjugated with D-quinic acid, giving rise to the substance class of chlorogenic acids (CGAs). Coffee beans contain high levels of CGAs and are therefore considered the richest dietary source of this substance class. CGAs have been shown to have several beneficial health properties, including antioxidant activity, anticarcinogenic and antidiabetic potential. However, their positive effects on human health highly depend on their bioavailability. In the present study, we investigated the intestinal absorption of hydroxycinnamates using the human colon carcinoma cell line Caco-2, serving as an *in vitro* model for intestinal absorption. Physiological conditions were modeled using the dynamic Ussing chamber model to simulate apical/basolateral compartments of the mucosa. The test substances (5-*O*-caffeoylquinic acid (5-CQA), caffeoylquinic acid lactones (CQLs), caffeic (CA) and ferulic acid (FA)) were added individually to the apical compartment of the chamber to physiological concentrations. Structure-dependent absorption was observed, resulting in higher absorption efficiency for FA (12.5 ± 1.0%; 100 μM) than CA (0.33 ± 0.13%; 100 μM). A low absorption rate for 5-CQA (0.1 ± 0.08%; 100 μM) was in line with the existing literature. CQA and CQL-isomers showed comparable absorption efficiencies at 100 μM (0.07 ± 0.02%). Concentration-dependent absorption of the more hydrophobic CQLs, as well as their precursor 5-CQA, was found to be non-saturable, indicating a passive diffusion process. Overall, our results indicated that the absorption rates of hydroxycinnamates are substantially influenced by their physicochemical properties.

1. Introduction

Hydroxycinnamic acids and their derivatives represent a subgroup of polyphenols. They are products of secondary plant metabolism, and therefore widely distributed in plants and plant-based foods (Scalbert & Williamson, 2000). Epidemiological studies have shown that *trans*-caffeic (CA) acid followed by ferulic acid (FA) are the predominant phenolic acids in Western diets (Ovaskainen et al., 2008). Conjugation of the free acids with D-(-)-quinic acid (QA) results in a group of esters known as chlorogenic acids (CGAs), which are mostly found in coffee beans and coffee beverages (Clifford, 1999). The amount of CGAs in coffee depends on the roasting degree (from very light to dark) as well as coffee type (*Coffea arabica* Cv. Bourbon, *C. arabica* Cv. Longberry, *Coffea canephora* Cv. Robusta). Considering these parameters (roasting degree and coffee type), Farah, De Paulis, Trugo, and Martin (2005) concluded that caffeoylquinic acid (CQA) (209–6541 mg/100 g of dry matter), feruloylquinic acid (FQA) (26–509 mg/100 g of dry matter) and diCQA (n.d.–951 mg/100 g of dry matter) are the most abundant CGAs in coffee. Free hydroxycinnamic acids have also been found in coffee but in lower concentrations (Clifford, 2000). Additionally, an intermolecular ester of CGAs, a quinide, has been detected but only in ground, roasted coffee and has also been shown to depend on the roasting degree (from very light to dark) and coffee type (*C. arabica* Cv. Bourbon, *C. arabica* Cv. Longberry, *C. canephora* Cv. Robusta) (Farah et al., 2005). The amounts of caffeoylquinic acid lactones (CQLs) and feruloylquinic acid lactones (FQLs) reportedly range from 80 to 392 mg/100 g and 5–42 mg/100 g of dry matter, respectively.

CGAs have been shown to exhibit several beneficial health properties, including antioxidant activity, anticarcinogenic and antidiabetic potential (Bakuradze et al., 2011; Tunnicliffe, Eller, Reimer, Hittel, & Shearer, 2011). Whether CGAs exert positive effects on human health highly depends on their bioavailability. In a study of ileostomists, absorption rates of 5-*O*-caffeoylquinic acid (5-CQA) were shown to be 33% compared to 95% for the free acid CA (Olthof, Hollman, & Katan, 2001). Further, an absorption rate of 8% 5-CQA was measured in participants lacking a colon (Erk et al., 2012), in contrast to 29% in healthy

* Corresponding author at: TU Kaiserslautern, Department of Chemistry, Division of Food Chemistry and Toxicology, Erwin-Schroedinger-Strasse 52, 67663 Kaiserslautern, Germany. Tel.: +49 631 205 2973; fax: +49 631 205 3085.
E-mail address: richling@chemie.uni-kl.de (E. Richling).

http://dx.doi.org/10.1016/j.foodres.2014.03.031
0963-9969/

volunteers (Stalmach, Steiling, Williamson, & Crozier, 2010). These findings indicate the significance of the colon on the bioavailability. Nevertheless, *in vivo* data alone do not allow quantification of the absorption rates of individual CGAs since once absorbed, they undergo diverse metabolic reactions, such as hydrolysis of esters, methylation as well as inter-esterification reactions between structural isomers (Stalmach et al., 2009). In former studies, such effects have typically been observed at pH > 6 (Farah, De Paulis, Moreira, Trugo, & Martin, 2006), as exemplified by studies of the upper gastrointestinal tract (GIT) (Hagl et al., 2011). All these transformations are likely to have an influence on the bioavailability, and thus hinder the acquisition of reliable data on structure–absorption relationships.

To date, only limited literature has been available on absorption rates or mechanisms as well as structure- and/or dose-dependent relationships of individual CGAs. In cultured gastric epithelial monolayers and the Ussing chamber model using ileal pig mucosa, different rates of absorption of several CGAs have been observed (Erk et al., 2014). Across pig mucosa, CGAs were found to be absorbed in the following order: diCQA (trace) < CQA < CA < FQA < QA. The crucial factors for absorption have been suggested to be the molecular weight, hydrophilicity and physicochemical properties of the subgroups, as well as the presence of stretchable and elastic bonds, such as in QA (Hagl et al., 2011). Mechanistic studies *in vitro* have indicated two modes of action for absorption. The main transport is *via* paracellular pathways by passive diffusion, although some compounds, *e.g.*, CA and FA, are partially transported by the monocarboxylate transporter (MCT) (Farrell, Dew, Poquet, Hanson, & Williamson, 2011; Konishi & Kobayashi, 2004). Additionally, dose–absorption relationships have been investigated using the Ussing chamber model with ileal pig mucosa (Deußer et al., 2013; Erk et al., 2014; Hagl et al., 2011) as well as Caco-2 cells (Konishi & Kobayashi, 2004). Non-saturable transport and a linear dose–flux relationship were observed for different CQAs at various concentrations. The Ussing chamber approach generally has the advantage that physiological conditions can be modeled by adjusting the pH in both compartments (apical: pH 6.0; basolateral: pH 7.4) and measuring the integrity of the cell monolayer by monitoring the transepithelial resistance over a period of 4 h. Recently, Poquet, Clifford, and Williamson (2008) have investigated the transport of FA across a cell monolayer of co-cultured Caco-2 and HT29-MTX cells and demonstrated the formation of several conjugates, such as feruloyl sulfate, feruloyl glucuronide, and hydroferulic acid. Studies by other groups supported these findings and further showed that the presence of a proton gradient enhances absorption (Konishi & Kobayashi, 2004; Konishi, Kobayashi, & Shimizu, 2003).

We here report results of an *in vitro* study in which intestinal absorption of hydroxycinnamates was investigated using the human colon carcinoma cell line Caco-2 in the Ussing chamber model, mimicking the functional and morphological organization of the intestinal epithelium. The main coffee polyphenols, CQA as well as the monomers CA and FA and for the first time CQLs, were added individually to the apical compartment of the chamber to concentrations equivalent to physiological gut lumen concentrations (0.1–1 mM). On the basis of reported polyphenol instability under cell culture conditions (Hong et al., 2002; Schaefer et al., 2006), we also investigated the stability of our selected hydroxycinnamates in the Ussing chamber incubation buffer.

2. Materials and methods

2.1. Chemicals

Unless otherwise indicated, all chemicals were of analytical grade. 5-CQA, CA, FA, 3,4,5-trimethoxycinnamic acid, the HPLC solvent (acetonitrile (MeCN), gradient grade) and Hanks' balanced salt solution (HBSS) were obtained from Sigma Aldrich (Steinheim, Germany). Methanol (MeOH) and hydrochloric acid (HCl) were purchased from J.T. Baker (Deventer, The Netherlands). Formic acid (HCOOH), dimethyl sulfoxide (DMSO) and $NaHCO_3$ were obtained from Merck (Darmstadt, Germany). Non-commercially available substances, such as 3-CQA, 3-CQL, were synthesized in-house as described below.

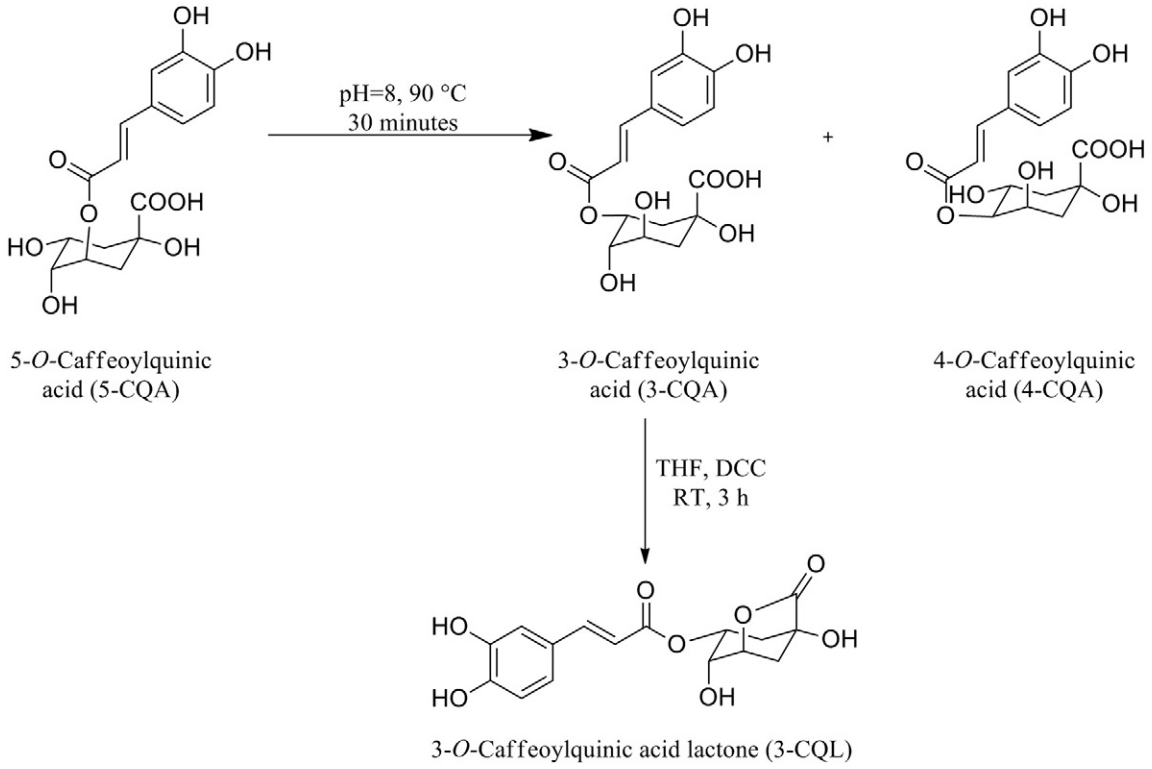

Fig. 1. Scheme of isomerization of 5-*O*-caffeoylquinic acid to 3- and 4-*O*-caffeoylquinic acid (modified according to Trugo & Macrae, 1984) as well as scheme for the synthesis of 3-*O*-caffeoylquinic acid lactone from 3-*O*-caffeoylquinic acid (modified according to Neises & Steglich, 1978).