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Aglycone structures and glycosylations affect anthocyanin transport and uptake in human gastric epithelial (NCI-N87) cells

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ABSTRACT

Anthocyanins are ubiquitous pigments in the human diet, but their metabolic fate is poorly understood. Absorption of these compounds may occur in the stomach, where acidic pH favors anthocyanin stability. The NCI-N87 cell line gastric model was used to examine the effects of anthocyanin chemical structure on their transport and uptake at pH 3, representative of the gastric environment. Chokeberry, containing different cyanidin derivatives, was used to determine the effects of different sugar substitutions; red grape was used as a source of monoglucosylated derivatives of different aglycones. The type of sugar substitution on cyanidin affected both cellular uptake and transport to the basolateral chamber, with cyanidin-3-arabinoside showing the greatest transport and cyanidin-3-glucoside the highest uptake by the cell. The aglycone structure also affected uptake and transport. Anthocyanins bearing B-ring di-substitution, cyanidin-3-glucoside and peonidin-3-glucoside, were transported and taken up the most by cells. Transport through NCI-N87 cells was most efficient for cyanidin-3-glucoside followed by peonidin, delphinidin, pelargonidin, malvidin, and petunidin-3-glucosides, in that order. Cyanidin-3-glucoside was also more efficiently taken up by the cells, followed by peonidin, pelargonidin, delphinidin, petunidin, and malvidin-3-glucosides. Although the metabolism of anthocyanins requires much more study, further evidence of the role of chemical structure is provided.

1. Introduction

Anthocyanins compose the largest group of water soluble naturally occurring pigments, with > 700 unique structures having been identified (Andersen and Jordheim, 2014). These natural pigments are gaining popularity as natural food colorants due to the wide variety of colors they impart, dependent on several structural and environmental factors, and also due to their potential health benefitting properties relating to antioxidant and anti-inflammatory activities (He and Giusti, 2010; Wallace and Giusti, 2014). Most studies have found the bioavailability of these compounds to be quite low; however recent works are finding them to be more bioavailable than previously believed (Kay et al., 2017). However, their metabolism within the body has not been well-characterized and is complicated by the wide diversity of anthocyanin chemical structures (Hertog et al., 1993; Prior and Wu, 2006).

Differentiation among anthocyanidins, anthocyanins lacking glycosylation, depends on varying methoxylations and hydroxylations on the chromophore; the 6 most commonly consumed anthocyanins show these variations on the B-ring, Fig. 1 (Andersen and Jordheim, 2006). Glycosylation, attachment of sugar moieties to the anthocyanidin through *O*-linkages, improves the stability of the pigment and also increases its water solubility (He and Giusti, 2010). The typical site of glycosylation is C_3, followed by C_5 and less commonly at C_7, $C_{3'}$, $C_{4'}$, and $C_{5'}$, Fig. 1 (Schwartz et al., 2008). A variety of sugars have been reported as attachments to anthocyanins from monosaccharides to trisaccharides (Andersen and Jordheim, 2006).

In humans, most nutritional absorption typically occurs in the small intestine; however, several studies suggested that significant amounts of anthocyanins are likely absorbed in the stomach (Fernandes et al., 2012; Passamonti et al., 2005, 2003, 2002; Talavera et al., 2005). It has been proposed that anthocyanins are absorbed through the gastric epithelium by the transport protein bilitranslocase (Passamonti et al., 2002). In rat liver plasma membrane vesicles, mono- and di-glycosides were better ligands for bilitranslocase than the corresponding anthocyanin aglycones (Passamonti et al., 2002). Decreasing anthocyanin polarity or increasing degrees of methoxylation on the B-ring increased affinity for bilitranslocase between anthocyanin monoglycosides (Passamonti et al., 2002). Most anthocyanin monoglyscosides acted as competitive inhibitors of bilitranslocase, but cyanidin-3-galactoside acted as a non-competitive inhibitor, with a significantly lower affinity

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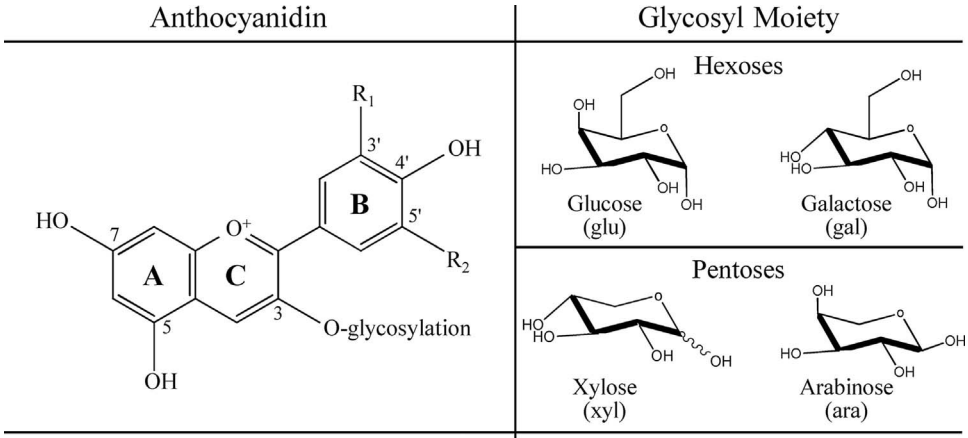

Anthocyanin	Abbr	R_1	R_2
Pelargonidin	Pg	H	H
Cyanidin	Cy	OH	H
Delphinidin	Dp	OH	OH
Peonidin	Pn	OCH_3	H
Petunidin	Pt	OCH_3	OH
Malvidin	Mv	OCH_3	OCH_3

Fig. 1. Basic structure of the most common anthocyanins and selected glycosyl moieties found in commonly consumed fruits and vegetables.

for the carrier. In a pig model, an 8-fold higher apparent absorption of pelargonidin compared to cyanidin was observed, measured by urinary output (Wu et al., 2004). Subsequently, cyanidin derivatives were found in higher quantities over delphinidin derivatives, and excretion of anthocyanin rutionsides was higher than that of glucosides (Wu et al., 2005).

Few options exist for *in vitro* gastric cell culture models capable of maintaining functionality at acidic pH, and of those, only 2 are human derived (Oliveira et al., 2015). In the gastric cell model MKN-28, a general time dependent transport of anthocyanins was observed (Fernandes et al., 2012). Numerically greater rate of absorption occurring at pH 3.0 was observed; however, there was no statistical difference in transport efficiency which warrants additional study (Fernandes et al., 2012). The effects of ethanol, pH (5.0 and 7.4), and glucose concentration on anthocyanin uptake were evaluated, and no differences were observed between delphinidin, cyanidin, and malvidin glucosides (Oliveira et al., 2015). The MKN-28 cell line was found to be contaminated by MKN-74 cells and is no longer being offered by many companies (Japanese Collection of Research Bioresources Cell Bank, 2015). Due to these issues of contamination or misidentification, the results from these studies may be questioned.

It is clear from these studies that both the aglycone structure and various common sugar attachments can play roles in the *in vivo* activity of anthocyanins; however the effects of chemical structure on the uptake of anthocyanins in the stomach have not been elucidated. Therefore the objective of this work was to investigate the role of chemical structure on the transport and uptake of anthocyanins by NCI-N87 cells, an *in vitro* model of the gastric epithelium. These studies were conducted at acidic apical pH to resemble common conditions in the gastric chamber and also because anthocyanin transport and uptake were generally favored at pH 3.0 with this cell line (Atnip et al., 2017). Previous work demonstrated pH and concentration to affect the transport and uptake of the total amount of anthocyanins (cyanidin-3-glycosides) (Atnip et al., 2017), not accounting for the roles of the of different glycosylation structures of the same aglycone or the different hydroxylation and methoxylation patterns of the 6 predominant anthocyanidins in acidic pH. In this study, chokeberry and red grape extracts were used as anthocyanin sources to allow for comparison of four major cyanidin-3-*O*-monoglycosides and six dietary anthocyanin aglycones, all with 3-O-glucoside attachments, to compare under identical treatment conditions.

2. Materials and methods

Unless otherwise indicated, all supplies and chemicals were purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). Cyanidin-3-glucoside chloride (Kuromanin chloride) was analytical grade (≥95% by HPLC) and purchased from Sigma Aldrich. Anthocyanin extracts were prepared from chokeberry (*Aronia melanocarpa*) juice concentrate, donated by Artemis International (Fort Wayne, IN), and from fresh red grapes (*Vitis vinifera)* obtained from a local market (Kroger, Columbus OH). Extracts were stored under dark freezing (–20 °C) conditions.

2.1. Extraction and purification of anthocyanins for cell culture assays

Chokeberry juice concentrate, courtesy of Artemis International (Fort Wayne, IN), was stored under dark refrigeration (4 °C) conditions. Anthocyanin-rich extracts were prepared following methods of Rodríguez-Saona and Wrolstad (2001) and purified by those of He and Giusti (2011). Briefly, anthocyanins were from plants materials with acidified 70% acetone; the extract was partitioned with chloroform, volume 2 × aqueous acetone extract. After overnight storage at 4 °C, the aqueous phase was collected, and residual acetone or chloroform was removed by rotary evaporation. The resulting extracts were then purified by cation exchange (MCX SPE cartridge, Waters Corp., Milford, MA). Anthocyanin content of the extracts was determined using High Pressure Liquid Chromatography (HPLC) as described below.

2.2. Cell culture conditions

The human gastric epithelial cell line NCI-N87 was purchased from the American Type Culture Collection (Manassas, VA). Growth conditions of the cells were replicated from previous work (Atnip 2014). Briefly, they were initially seeded in 75-cm^2 culture flasks (Corning, Corning, NY) with RPMI-1640 complete medium supplemented with 10% fetal bovine serum, 100 µg/mL penicillin, and 100 µg/mL streptomycin. Cells were grown at 37 °C under 5% CO_2, then seeded on