Computational Biology and Chemistry 56 (2015) 152–158

Contents lists available at ScienceDirect

Computational Biology and Chemistry

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

Research Article

Study on the interaction of artificial and natural food colorants with human serum albumin: A computational point of view

Diego Masone [a,*], Céline Chanforan [b]

[a] CONICET, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre Jorge Contreras 1300, Mendoza 5500, Argentina
[b] Chr. Hansen France SAS, 92 avenue des Baronnes, 34730 Prades-le-lez, France

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 13 March 2015
Accepted 16 April 2015
Available online 20 April 2015

Keywords:
Food colorants
Human serum albumin
Docking
Molecular dynamics

ABSTRACT

Due to the high amount of artificial food colorants present in infants' diets, their adverse effects have been of major concern among the literature. Artificial food colorants have been suggested to affect children's behavior, being hyperactivity the most common disorder. In this study we compare binding affinities of a group of artificial colorants (sunset yellow, quinoline yellow, carmoisine, allura red and tartrazine) and their natural industrial equivalents (carminic acid, curcumin, peonidin-3-glucoside, cyanidin-3-glucoside) to human serum albumin (HSA) by a docking approach and further refinement through atomistic molecular dynamics simulations. Due to the protein–ligand conformational interface complexity, we used collective variable driven molecular dynamics to refine docking predictions and to score them according to a hydrogen-bond criterion. With this protocol, we were able to rank ligand affinities to HSA and to compare between the studied natural and artificial food additives. Our results show that the five artificial colorants studied bind better to HSA than their equivalent natural options, in terms of their H-bonding network, supporting the hypothesis of their potential risk to human health.

1. Introduction

In food manufacturing the addition of color is almost ubiquitous to improve consumers' perception of the processed products. Indeed, the addition of food colors allows insuring a standardized shade of food products. Today, natural colors are largely used as food ingredients in Europe and in the US, whereas in other regions, such as South America, artificial colors (ACs) are still considered the main solution for food coloring purposes (Hallagan et al., 1995; Wissgott and Bortlik, 1996), (for more details please see Global New Products Database 2014, www.gnpd.com). Although processed food might power human evolution (Kim, 2013), current levels of ACs in the acceptable daily intake are of major concern. Not surprisingly, the amount of ACs certified by the Food and Drug Administration of the United States has increased from 12 mg/capita/day in 1950 to 68 mg/capita/day in 2012 (Stevens et al., 2013a). Moreover, industrialized food designed to be consumed by infants may contain the highest concentrations of food colorants, either natural or artificial (Hofer and Jenewein, 1997). Consequently, the effects of these substances on human health have been extensively questioned in the literature (Abbey et al., 2014; Bolel et al., 2012; Stevens et al., 2013a,b, 2011; Arnold et al., 2012; Weiss, 2011). In particular, several studies on children behavioral problems related with ACs emphasize attention problems, hyperactivity, irritability, sleep disorders and aggressiveness (Stevens et al., 2013a; McCann et al., 2007; Bateman et al., 2004).

In Fig. 1 ligand molecules are shown. Tartrazine is an artificial synthesized acid azo dye which is water soluble and yellow in solution (Li et al., 2014). Recent studies on rats pointed out that tartrazine and carmoisine (also known as azorubine) alter biochemical markers in vital organs such as liver and kidney, not only at high doses but also at lowest ones (Amin and Elsttar, 2010). While other studies demonstrated the adverse effects of tartrazine in learning and memory functions (Gao et al., 2011), as well as on the male reproductive function (Mehedi et al., 2009).

Allura red is a water-soluble, monoazo class of synthetic food pigment, with extraordinary stability in many manufactured food products such as candy coating, ice creams, drinks and confectionery (Wang et al., 2014). Allura red's side effects have been reported experimentally (Chung, 2000) and include DNA damage in male mice (Tsuda et al., 2001). Quinoline yellow is a synthetic colorant with possible genotoxic characteristics, as suggested from two different cellular model systems, human lymphocytes

* Corresponding author. Tel.: +54 2614236003.
E-mail address: dmasone@fing.uncu.edu.ar (D. Masone).

http://dx.doi.org/10.1016/j.compbiolchem.2015.04.006
1476-9271/

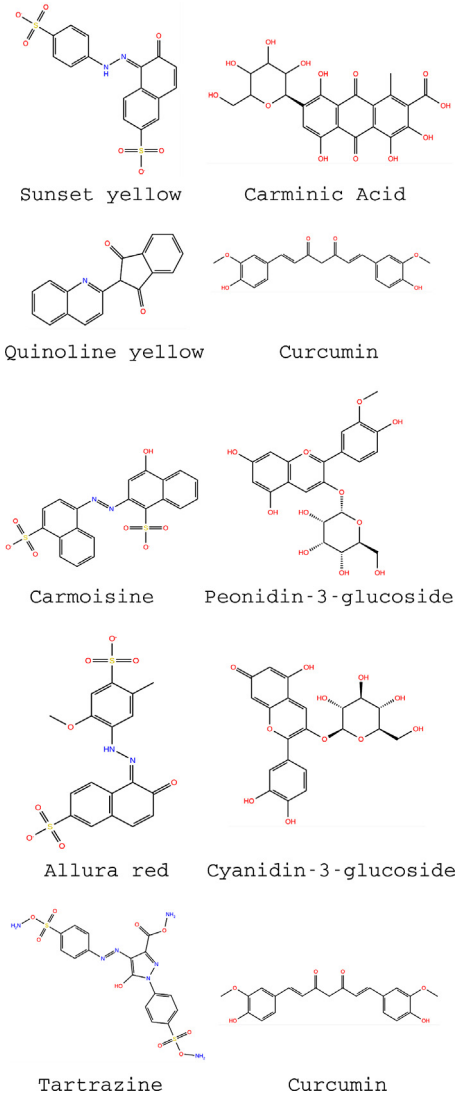

Fig. 1. Selected artificial food colorants (left) with their respective natural equivalents (right).

in vitro and *Vicia faba* root-tip meristems, in vivo (Macioszek and Kononowicz, 2004). While sunset yellow has shown reproductive and neurobehavioral effects (Tanaka, 1996) as well as hyperactivity (Ward, 1997).

In some food applications, such as confectionery and beverages, yellow ACs quinoline or tartrazine may be matched by their natural equivalent: curcumin (diferuloylmethane) (Hallagan et al., 1995; Aggarwal et al., 2007). This pigment is extracted from the rhizome of *Curcuma longa*, and may also be used as natural flavor in food products. However, due to its light sensitivity and pH dependency, yellow ACs might be preferred by the food industry (Batista et al., 2006). Another yellow orange AC is sunset yellow which is matched in food applications by a natural pigment extracted from the cochineal insect (*Dactylopius coccus*): carminic acid, (Koren, 1994). Allura red and carmoisine exhibit red and pinkish shades, respectively, when applied in foods. Anthocyanins are generally used as a natural solution for the replacement of these ACs. Natural colors based on anthocyanins consist of fruits or vegetables extracts, and their shade is pH dependent. These pigments, also used as ingredients in the pharmaceutical industry, contain antioxidants and have shown positive health effects (Tsuda, 2012; He and Giusti, 2010; Sarni-Manchado and Cheynier, 2006).

Table 1
Food colorants to be used in processed food allowed by the Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. For artificial ones the labeling shall mention: may have an adverse effect on activity and attention in children.

Food colorant	E number in EU	Natural equivalent	E number in EU
Sunset yellow	E 110	Carminic Acid	E 120
Quinoline yellow	E 104	Curcumin	E 100
Carmoisine	E 122	Peonidin-3-glucoside	E 163
Allura red	E 129	Cyanidin-3-glucoside	E 163
Tartrazine	E 102	Curcumin	E 100

Aware of possible children's health issues related to the consumption of ACs, the European Parliament published a list of artificial food colors allowed to be used in processed food but requiring a warning label on food packaging (see Table 1). This modification of the European regulation is aligned with the evolution of European and North American customers perception over the last years; indeed many customers are looking for food products with "all natural" claims on packaging (Bartels and Onwezen, 2014; Tully and Winer, 2014; Hsu and Chen, 2014).

During the last decade atomistic simulations have been able to contribute in the understanding of critical problems in biophysics and computational chemistry, such as protein folding, protein–protein and protein–ligand docking (Piana and Laio, 2007; Pietrucci et al., 2009; Berteotti et al., 2009). These advances stand on the shoulders of newly developed methods that explore free energy landscapes more efficiently, together with better and faster computer processors (Van Der Spoel et al., 2005). Remarkably, collective variable driven molecular dynamics (MD) have shown to suitably sample complex conformational changes in biomolecules by accelerating rare events not observable by classical MD (Fiorin et al., 2013). In particular, restrained MD simulations are able to surpass intrinsic limitations of the physical models, resulting in a more efficient statistical sampling (Fiorin et al., 2013; Laio and Parrinello, 2002; Laio and Gervasio, 2008; Kumar et al., 1996). Besides, considering that large computer power is not available to all, the problem of rapidly obtaining realistic dynamic information on proteins remains an issue of principal interest (Cossins et al., 2012).

In this study we analyze by computational means how 5 artificial food colorants and their 4 natural equivalents interact with human serum albumin (HSA), the most abundant protein in plasma which contributes to about 80% of the blood osmotic pressure (He and Carter, 1992; Peters, 1995). Together with alpha-1-acid glycoprotein, HSA is responsible for transporting drugs, steroids, bilirubin, thyroid hormones, fatty and colic acids (Zunszain et al., 2003; Peters, 1995; Honoré, 1990), as it contains two structurally selective binding sites (Jisha et al., 2006). The binding of such artificial and possibly toxic compounds to HSA is of physiological relevance, since binding to HSA can be the way to control these substances concentrations as well as their side effects (Pan et al., 2011). As Basu and Kumar recently pointed out, the available free concentration for toxic action can be regulated by high binding to serum proteins (Basu and Kumar, 2014). The binding between ACs and HSA could then affect the absorption, distribution, metabolism and toxicity of these compounds and may alter the functions and structure of the receptor protein (Wang et al., 2014).

2. Computational methods

2.1. Docking

In order to locate the protein binding site of each one of the 9 ligands (4 natural colorants and 5 artificial ones, since curcumin