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Stabilising phycocyanin by anionic micelles

Mia Fiilsøe Falkeborg [a,*], Maria Cinta Roda-Serrat [b], Kim Lolck Burnæs [a], Anne Louise Dannesboe Nielsen [a]

[a] Centre for Food Technology, Danish Technological Institute, Kongsvang alle 29, DK-8000 Aarhus C, Denmark
[b] Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

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ABSTRACT

Phycocyanins are pigment-protein complexes with potential application as natural food colourants. The perceived colour of phycocyanins varies with pH, and a method to stabilise the colour over a broad range of pH values is requested by the food industry. In this work, the stabilising effect of sodium dodecyl sulphate (SDS) micelles on pH-induced colour variations of phycocyanin was examined. SDS was shown to stabilise the blue conformation of phycocyanin, preventing formation of the green conformation, which is prevalent at low pH. The studies indicated that the stabilising effect occurred through interaction or entrapment of the non-protonated, circular helical (blue) structure of phycocyanin and the anionic SDS micelles. The interaction prevented conversion into protonated, partially unfolded (green) phycocyanin species. This information opens for new possibilities to stabilise the blue conformation of phycocyanin and to apply the stabilised form in food products as a natural blue food colourant.

1. Introduction

The range of available blue, green, and purple shaded natural pigments for use in food is limited, in contrast to red, yellow, and orange natural pigments, which are relatively easy to produce with colourants such as carmine, carotenoids, and anthocyanins (Pina, Melo, Laia, Parola, & Lima, 2012). The food and beverage industry is seeking naturally derived blue-shaded colourants to replace the chemically produced colourants currently in use (Jespersen, Strømdahl, Olsen, & Skibsted, 2005; Newsome, Culver, & Breemen, 2014). In this context, phycocyanins have received attention. Phycocyanins are water-soluble light-harvesting proteins found in cyanobacteria. Three types of bilins are found attached to phycocyanins – phycocyanobilin, phycobiliviolin, and phycoerythrobilin (Bermejo, 2014). Phycocyanobilin is an open-chain tetrapyrrolic compound, which, in native phycocyanins, gives rise to an absorbance maximum at 590–625 nm, making it a useful blue pigment for use as a natural food colourant (Bermejo, 2014; Newsome et al., 2014).

The application of phycocyanin in food is limited by its instability towards changes in the surrounding matrix. As shown by Jespersen et al. (2005), phycocyanin is unstable towards heat and light in aqueous solution and the protein moiety denatures at temperatures above 45 °C, leading to changes in colour. Instability related to protein stability (protein denaturation) and/or microbial spoilage can be improved by formulation engineering, e.g. by using additives such as trehalose, citric acid (DIC Lifetec Co., 2012) or glycerol (Pottecher, 2015). However, the protonation state of phycocyanobilin changes its absorbance spectrum, making the colour of the pigment sensitive to changes in acidity. Detailed modelling of the chromophores of phycocyanins were made by Stanek and Grubmayr (1998), who showed that protonation of the chromophore led to a wavelength shift in its absorbance spectrum. This is a challenge for the application of phycocyanins in food, as the perceived colour of the pigment shifts from blue shades around neutral pH, to green shades at lower pH. Similar pH-dependent variations in perceived colour have been observed for anthocyanins. Anthocyanins represent a wide group of natural pigments, the colours of which depend greatly on the surrounding pH and hence the protonation state of the molecules (Moreira et al., 2003; Pina et al., 2012). Several reports have shown that anthocyanins can be stabilised by interaction with micelles formed from e.g. sodium dodecyl sulphate (SDS) (Lima et al., 2002; Liu, Fu, & Nian, 2014; Mulinacci et al., 2001). These reports conclude that the colour stabilisation occurs through interaction of the positively charged flavylium cation of anthocyanins with the negatively charged SDS micelle.

A method for colour stabilisation based on encapsulation of phycocyanin in multiple emulsions has been patented (Bonnet & Mason, 2012), however, this method requires a substantial amount of encapsulation material, and the final product is thus very low in phycocyanin concentration. Furthermore, the products cannot be used in transparent products, and the stabilisation towards pH-

* Corresponding author.
E-mail address: mfa@dti.dk (M.F. Falkeborg).

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dependent colour variations is only effective until the pH of the inner water-emulsion becomes adjusted to the pH of the outer solution (the food).

In order to use phycocyanin as a blue food colour, the colour needs to be stable over a wide range of pH values, in particular acidic and neutral pH values, as these are common for food. Inspired by the work done with anthocyanins and SDS micelles, the aim of this work was to evaluate whether SDS micelles are capable of stabilising the blue colour of phycocyanins over a range of food-related pH values. The work is based on experimental findings regarding the effect of pH and SDS concentration on phycocyanin as well as phycocyanobilin, coupled to data about conformational structures of phycocyanobilin reported in literature. The work has resulted in new hypotheses for a mechanism to stabilise phycocyanin against pH-dependent colour variation; a mechanism, which could be the key to develop a stabilised phycocyanin-based blue food colour.

2. Materials and methods

2.1. Materials

Phycocyanin was supplied as the commercial product Linablue G1 from DIC Spirulina (Chuo-ku, Tokyo 103–8233, Japan). This formulation contained 40% Spirulina colour, including phycocyanin and other proteins, 5% tri-sodium citrate, and 55% D-trehalose. The same lot of Linablue was used throughout the work. Citric acid (Sigma-Aldrich) in demineralised water was used to prepare standard aqueous solutions with pH 6, 5, 4, 3, 2.5, 2, and 1.9, all ± 0.1. The final pH was measured using a Metrohm 827 pH lab. SDS, and sodium sulphate (Na_2SO_4) were from Sigma-Aldrich.

2.2. Effect of pH and SDS on phycocyanin

Aqueous solutions of phycocyanin (1% w/w Linablue in demineralised water) and varying amounts of SDS (1.1, 0.7, 0.1, 0.05, 0.01, or 0% w/w) were prepared. One mL phycocyanin-SDS solution was combined with 5 mL pH-solution, according to the experimental design presented in Table 1. All solutions were prepared fresh each time.

2.3. Effect of pH and SDS on phycocyanobilin

Denatured phycocyanin was obtained by washing Linablue with methanol at room temperature in the proportion 8 ml methanol/g Linablue. Phycocyanobilin was cleaved from denatured phycocyanin by reflux alcoholysis as detailed by O'Carra and O'hEocha (1966). Evaporation of the reaction mixture was performed in a Rotavapor R-210 (Buchi).

Aqueous solutions of phycocyanobilin of 0.001% w/w were prepared and different volumes of SDS (100 mM) were added to the solutions to achieve the desired SDS concentrations. The final concentration of phycocyanobilin was 0.0009 ± 0.00007% w/w. All solutions were prepared fresh each time.

2.4. Analysis methods

For each solution of phycocyanin or phycocyanobilin, with varying SDS concentrations at varying pH values, the absorbance spectrum was recorded from 700 nm to 300 nm in absorbance mode with 1 nm-intervals in a Perkin Elmer Lambda 25 UV/VIS spectrophotometer. The concentration of Linablue chosen for this study (1% w/w) allowed the samples to be analysed directly without dilution. Only phycocyanin and phycocyanobilin absorbed light is this region, and SDS, citric acid, trehalose or citrate did not interfere with the absorbance. In all experiments in this work, the order of mixing phycocyanin, SDS and/or citric acid solutions, did not influence the results.

The visible-ultraviolet ratios (A_{VIS}/A_{UV}) were calculated by dividing the intensity of the absorbance spectra at its maximum in the visible region (~600 nm) by the intensity of the maximum in the ultraviolet region (~360 nm). The L*a*b* values were calculated through colour analysis, which was made from the visible spectra data with the CIE 1931 2° Standard Observer and the CIE standard illuminant D65, following the CIE 1976 (L*a*b*) colour space CIELAB (Commission Internationale de l'Eclairage, 15:2004). Differences in L*, a*, and b* values were notated ΔL, Δa, and Δb, respectively, and the total difference between samples (distance on the CIELAB diagram) was notated ΔE and calculated according to Eq. (1).

$$\Delta E = ((\Delta L^2) + (\Delta a^2) + (\Delta b^2))^{\frac{1}{2}} \quad (1)$$

2.5. Thermo-stabilising effect of SDS

One mL aqueous phycocyanin solution (1% w/w Linablue in demineralised water), with or without 0.7% w/w SDS, was mixed with 5 mL standard pH-solution at pH 2 and pH 6. The absorbance spectrum for each solution was measured before and after incubation for one hour in a waterbath at 65 °C, and the L*a*b* values and change in total colour (ΔE) were calculated. The results are presented as the average of three analyses.

2.6. Influence of ionic strength

An aqueous solution of phycocyanin (1% w/w Linablue in demineralised water) was prepared containing 0.54% w/w Na_2SO_4. This amount of Na_2SO_4 corresponds to the molar concentration of SDS at 1.1% w/w (the highest evaluated in this study) and hence provides similar ionic strength as SDS at this concentration. One mL of the solution was combined with 5 mL standard pH-solution at pH 2 and pH 6. The absorbance spectrum for each solution was measured and compared to absorbance spectra for similar samples with no Na_2SO_4. The L*a*b* values and change in total colour (ΔE) were calculated, and the results are presented as the average of two analyses.

Table 1
Experimental design. Aqueous phycocyanin solutions (1%) with SDS concentrations 0-1.1.0% (1 mL total) were combined with pH standard solutions (5 mL) at values marked with "•".

		pH standard solution						
		1.9	2.0	2.5	3.0	4.0	5.0	6.0
SDS%	1.10		•				•	
	0.70	•	•	•	•	•	•	•
	0.35		•				•	
	0.10		•				•	
	0.05		•				•	
	0.01		•				•	
	0	•	•	•	•	•	•	•