

Anthocyanin–aluminium and –gallium complexes in aqueous solution

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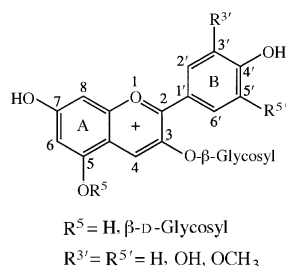
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Complexation of aluminium and gallium ions with synthetic anthocyanin models and natural anthocyanins extracted from the blue flowers of *Evolvulus pilosus* cv 'Blue Daze' and the violet flowers of *Matthiola incana* has been thoroughly investigated in aqueous solution. From UV–VIS spectroscopic data collected at pH 2–5, the presence of complexes, involving not only the coloured forms but also the colourless forms of the pigments is demonstrated. A theoretical treatment is developed for the calculation of the corresponding stability constants. The pigments studied throughout this work can be divided into two series, one sharing a cyanidin chromophore and the other a delphinidin one. Within both series, individual pigments are distinguished according to the degree and type of glycosylation and/or acylation. Intramolecular effects such as copigmentation of anthocyanin–aluminium complexes and the effect of the presence of a malonyl group on the formation of those complexes are discussed. These results are important to plant pigmentation and, for instance, a narrow pH domain in which colour amplification due to complexation is at a maximum has been found.

Introduction

The chromophore units of anthocyanins are hydroxy- and methoxy-derivatives of the 2-phenylbenzopyrylium (flavylium) structure. They occur as non-plastid, water soluble glycosylated pigments dissolved in the vacuolar cell sap, predominantly in the epithelial tissue of flowers and fruits, more rarely in stems and leaves.^{1,2} These hydroxy- and methoxy-flavylium derivatives exist as glycosides of hydroxy groups (mainly at the 3 and 5 positions) of the benzopyrylium core. The glycosylating sugars may be mono-, di- or tri-saccharides, and the aglycone forms are known as anthocyanidins (Scheme 1). Additionally, anthocyanins may be acylated through esterification of the sugar residues with one or more of a variety of aliphatic acids, phenolic benzoic acids or phenolic cinnamic acids.



Scheme 1 The flavylium form of common natural anthocyanins

The more common anthocyanins (3-monoglucosides and 3,5-diglucosides), rapidly fade when put in mildly acidic aqueous solutions. Indeed, the water molecule readily reacts at position 2 of the flavylium cation with the consequent formation of large amounts of colourless forms (hemiacetal and chalcones) according to a reversible process called the hydration reaction (Scheme 2). *In vivo*, these pigments may be found in association with metal ions, other flavonoids (which may themselves be glycosylated and acylated) and probably with polysaccharide macromolecular carriers. All these interactions affect the

absorption spectra of the anthocyanins involved and thus they give access to the mechanisms explaining the great variations in plant colours. It is now well known that colourless polyphenols (flavones, flavonols, cinnamic and benzoic acid esters, tannins) are able to form molecular, non-covalent, stacking complexes with the large planar, π -electron rich flavylium nucleus. We have demonstrated that the hydrophobic interaction efficiently protects the chromophore against nucleophilic attack from water, displacing at the same time the overall equilibrium between the coloured and colourless forms towards the selectively complexed coloured forms.^{3–7} This phenomenon, called copigmentation, can also operate in an intramolecular way in more complex anthocyanins bearing cinnamic or benzoic acid residues on their glycosyl groups, leading to stable coloured solutions at mildly acidic pH values.^{2,8,9,17}

Unlike polyphenolic copigments, metal ions which could be present in the anthocyanin natural media seem more rarely involved in colour stabilization. However, small highly charged metal ions such as Al³⁺ and Mg²⁺ have been reported possibly to strengthen the pigment–copigment interaction leading to hyperchromic and bathochromic shifts. In particular, it has been proposed that the blue colour displayed by some flowers is the result of pigment–copigment–metal ion assemblies.^{3,21,22} The most remarkable achievement in that field is the X-ray structure of the commelinin pigment elucidated by Goto and Kondo, which shows up to six pigment and six copigment molecules packed around two magnesium ions in a crystalline state.²

A first report¹⁰ on anthocyanin–aluminium complexation has provided information on the mechanism of complexation. From UV–VIS spectroscopic measurements on equilibrated solutions at different pH values and relaxation kinetics measurements (pH-jump), the binding constants have been calculated and the percentage of different free and complexed pigment forms plotted as a function of pH. ¹H NMR analysis in CD₃OD (in which complexation is much stronger than in water), has confirmed the conversion of the anthocyanin from the red flavylium form to the deep-purple quinonoidal forms upon coordination to Al³⁺.