BRIEF COMMUNICATIONS

Structure of the blue cornflower pigment

Packaging red-rose anthocyanin as part of a 'superpigment' in another flower turns it brilliant blue.

The same anthocyanin pigment makes roses red but cornflowers blue[1], a phenomenon that has so far not been entirely explained. Here we describe the X-ray crystal structure of the cornflower pigment, which reveals that its blue colour arises from a complex of six molecules each of anthocyanin and flavone, with one ferric iron, one magnesium and two calcium ions. We believe that this tetrametal complex may represent a previously undiscovered type of supermolecular pigment.

An anthocyanin pigment was first isolated in 1913 as a red oxonium salt from the blue cornflower *Centaurea cyanus*[2], and later the same pigment was discovered in a red rose[3]. The colour variation was variously ascribed to a difference in flower-petal pH (ref. 3), an association of the pigment with metal ions[4] or with another pigment[5]. However, the blue-cornflower pigment (later named protocyanin[6,7]) was found to contain iron and magnesium[8,9] in complex with anthocyanin and flavone[10,11]; calcium is also essential for the reconstruction of protocyanin[12].

We determined the crystal structure of reconstructed protocyanin at 1.05 Å resolution. (For methods, see supplementary information.) In the refined molecule, the four metal ions Fe^{3+}, Mg^{2+} and two Ca^{2+} align along the pseudo-three-fold axis (Fig. 1a) and are coordinated to six anthocyanin (cyanidin 3-*O*-(6-*O*-succinyl glucoside)-5-*O*-glucoside) and six flavone (apigenin 7-*O*-glucuronide-4′-*O*-(6-*O*-malonyl-glucoside)) molecules. The inner Fe^{3+} and Mg^{2+} ions are each coordinated to three anthocyanin molecules, respectively, and the two outer Ca^{2+} ions are each coordinated to three flavone molecules (see supplementary information).

The two sites of the central nuclei have the same electron density, which corresponds to the average for Fe^{3+} and Mg^{2+} (results not shown). This suggests that the sites are occupied by either Fe^{3+} or Mg^{2+} because of the random orientation of the molecule along the pseudo-three-fold axis. To verify this, we investigated the X-ray structures of metal-substituted FeMgBa-, FeMnBa- and FeCdBa-protocyanins (results not shown). (Barium derivatives are more stable than calcium derivatives for isolation as crystals.)

In the FeMgBa-protocyanin, the electron density of the inner nuclei is almost the same as in protocyanin, whereas in the FeMnBa- and FeCdBa-protocyanins it is the average of Fe^{3+} and of the substituted ions, respectively. The distances between the two metals and the coordinating oxygen atoms vary according to the radii of the substituted metals. These results indicate that the two metals are heterogeneous. We conclude that the inner nuclei of protocyanin consist of Fe^{3+} and Mg^{2+} ions.

In the protocyanin molecule, the anthocyanins and flavones self-associate in pairs. The Fe^{3+} and Mg^{2+} ions are coordinated to different anthocyanin fragments in an anthocyanin pair (Fig. 1b, left); the two Ca^{2+} ions are coordinated with separate flavone fragments in a flavone pair (Fig. 1b, centre). Stacking of anthocyanin and flavone (co-pigmentation) is also evident (Fig. 1b, right). The C–C and C–O bond lengths in the B-ring of anthocyanin (data not shown) indicate that the anthocyanin is in the 4′-keto-quinoidal form.

Blue flower colours are caused mainly by delphinidin-type anthocyanins — for example, commelinin[13]. In protocyanin, however, the blue colour is produced by a cyanidin-type anthocyanin. The chelation of Fe^{3+} and Mg^{2+} with the 4′-keto-quinoidal base of anthocyanin is important for the blueing in protocyanin. The two Ca^{2+} ions coordinate with the flavones to form components that bring about co-pigmentation as well as stabilization of the molecule. The blue colour in protocyanin is therefore developed by a tetranuclear metal complex, which may represent a new type of supermolecular pigment.

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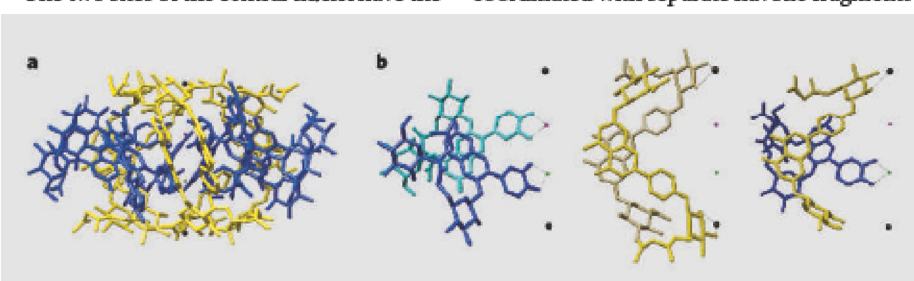

Figure 1 | Crystal structure of the protocyanin molecule. **a**, Side view along the pseudo-three-fold axis. Blue, anthocyanin; yellow, flavone glycoside; spheres: red, Fe^{3+}; green, Mg^{2+}; black, Ca^{2+}. For stereo view, see supplementary information. **b**, Side views of stacking pigment pairs. Left, one anthocyanin molecule binds Fe^{3+} (red), the other binds Mg^{2+} (green); centre, two flavones each bind to one Ca^{2+} (black); right, flavone and anthocyanin molecules bind to Ca^{2+} and Mg^{2+}, respectively.

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Supplementary information accompanies this communication on *Nature*'s website.
Competing financial interests: declared none.
doi:10.1038/436791a