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MAGNESIUM IN THE BLUE PIGMENT COMPLEX COMMELININ*

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Key Word Index—*Commelina communis*; Commelinaceae; commelinin; magnesium; metallo-anthocyanin; flower pigment.

Abstract—In the *in vitro* synthesis of commelinin from its component parts, awobanin, flavocommelin and Mg, the yield of commelinin was shown to be proportional to the amount of Mg added and commelinin was not obtained in the absence of Mg. The stabilities of commelinin and other metal complexes (Zn, Cd, Ni, Mn and Co-commelinins) in acidic solutions (pH 2.4–5.2) were different from one another according to the metal present. Of the six blue complex pigments, Ni- and Mg-commelinins were most stable, whereas Cd-commelinin was very unstable. On dialysis commelinin was impermeable and even after dialysis *ca* 80% of Mg in the pigment still remained. These facts indicate that Mg plays a part in the formation of the stable blue complex commelinin.

INTRODUCTION

The blue pigment commelinin from flowers of *Commelina communis* has been shown to be composed of three components, awobanin (delphinidin 3-*p*-coumaroyl glucoside 5-glucoside), flavocommelin (swertisin 4′-glucoside) and Mg by a series of experiments, i.e. component analyses of pure commelinin from nature [1–5] and *in vitro* synthesis of commelinin from three components [6]. Recently however, Goto *et al.* [7] reported the formation of commelinin from a mixture of awobanin and flavocommelin, throwing doubt on the participation of Mg in the formation of commelinin molecule. This investigation was undertaken to obtain further information on the role of Mg in this blue complex pigment.

RESULTS AND DISCUSSION

The effect of Mg *on the formation of the stable blue complex commelinin*

To mixtures of awobanin and flavocommelin (1:2 in mole ratio), magnesium acetate was added in various mole ratios (0–1.5). After repeated evaporation of the solution and re-solution of the residue in water as described previously [6], commelinin formed in each reaction mixture was separated from other material by passing through a Sephadex column and quantitatively determined spectrophotometrically. As already described by us [6], commelinin was not obtained without Mg. Moreover, as shown in Fig. 1, the yield of the blue complex commelinin changed according to the amount of Mg added. The yield of commelinin increased in proportion to the amount of Mg. At the approximate Mg/awobanin ratio of 0.5, which corresponds to the mole ratio in natural commelinin [awobanin : flavocommelin: Mg (2:2:1)], the amount of commelinin formed reached a maximum. These results indicate that Mg is essential in the formation of the stable blue complex. Mg may play a role for attracting awobanin-flavocommelin complexes as proposed by Goto *et al.* [7].

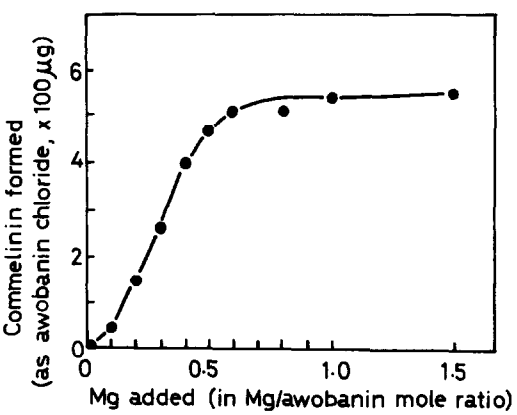

Fig. 1. The effect of Mg on the formation of the blue complex molecule of commelinin. Values are taken from the averages of five separate experiments.

The stability of commelinin and the magnesium replaced blue complexes in acidic solution

In order to obtain further information on the role of metals in commelinin, other metal complexes, i.e. Ni-, Cd-, Zn-, Mn- and Co-commelinins were synthesized [8] and the pH dependence of the absorption spectra of the blue pigments was examined in buffer solutions of different pH and in diluted HCl (0.1% and 1%). As shown in Fig. 2, Mg-commelinin (both natural and synthetic) showed a stable blue colour even at pH 2.4 and exhibited two absorption bands, Band I (λ_{max} 591 nm) and Band II (λ_{max}

* Part 4 in the series of "Metallo-anthocyanins". For Part 3, see ref. [9].

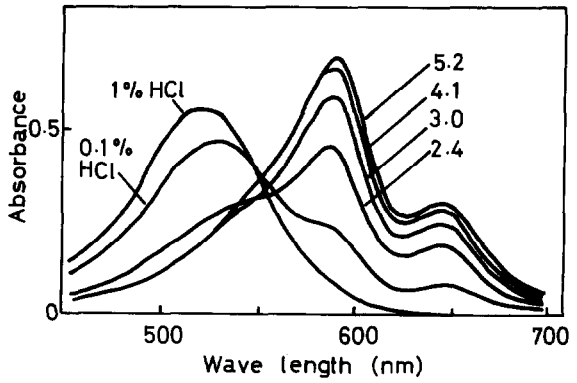

Fig. 2. Absorption spectra of Mg-commelinin in 0.05 M citrate–phosphate buffer and in dilute HCl. Numbers indicate pH values of the solutions.

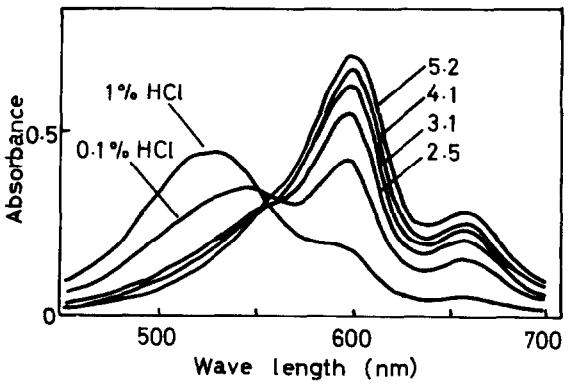

Fig. 4. Absorption spectra of Ni-commelinin in 0.05 M citrate–phosphate buffer and in dilute HCl. Numbers indicate pH values of the solutions.

647 nm), in the visible region, while in diluted HCl the solution changed to red and the absorption of the flavylium cation appeared, λ_{max} 532 nm in 0.1% HCl and λ_{max} 523 nm in 1% HCl respectively. In contrast, Cd-commelinin was very unstable in acidic solution (Fig. 3). At pH 3.1, the absorbances of its two bands decreased considerably and at pH 2.5, the solution became red and showed one absorption maximum at 532 nm, which was shifted to 523 nm in 1% HCl. The most stable blue complex of the six was Ni-commelinin as shown in Fig. 4, in which two bands were still clearly observed even in 0.1% HCl. Thus, the stabilities of the six blue complexes in acidic solutions varied according to the metal involved in complex formation (Fig. 5). These differences in stability as well as the spectral differences [8, 9] according to the metal involved confirm the view that metals are part of these complex molecules.

Dialysis of commelinin with a cellophane membrane

On dialysis, commelinin was impermeable through a cellophane membrane as described previously [1, 3]. The absorption spectrum of the dialysed pigment was actually identical with that of untreated commelinin. As shown in Table 1, *ca* 80% of Mg in the pigment still remained after

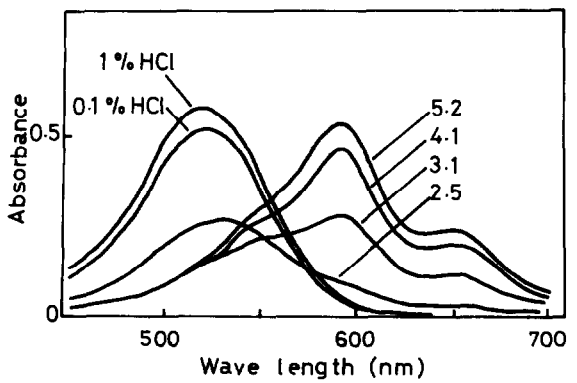

Fig. 3. Absorption spectra of Cd-commelinin in 0.05 M citrate–phosphate buffer and in dilute HCl. Numbers indicate pH values of the solutions.

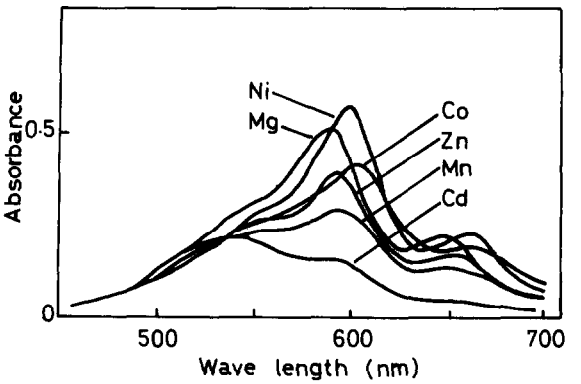

Fig. 5. Absorption spectra of the blue complex pigments, Mg-, Ni-, Cd-, Zn-, Mn- and Co-commelinins, in 0.05 M citrate–phosphate buffer, pH 2.6.

Table 1. Mg/awobanin mole ratio in the dialysed commelinin

Sample used (mg)	Mg/awobanin mole ratio
6.6	0.37
9.1.	0.37
20.9	0.38
8.3*	0.38
Mean	0.38
Untreated commelinin	0.47

*Mg acetate (1.9 mg) was added to the sample before dialysis.

dialysis. Under the same conditions, a sample to which magnesium acetate was added before dialysis gave almost the same result. Since commelinin is gradually decomposed in aqueous solution [10], a decrease of Mg may be attributable to the decomposition of the blue commelinin molecule. These facts show that Mg in the commelinin

molecule is scarcely ionized in its aqueous solution. The results obtained here indicate that the metal in commelinin plays an essential role in the formation of its stable blue complex.

EXPERIMENTAL

Anthocyanin and flavocommelin. Awobanin chloride was prepared from a crystalline specimen of commelinin as before [6]. Isolation and purification of flavocommelin were made according to the method reported previously [4].

Formation of the blue complex pigment from awobanin, flavocommelin and Mg. The quinoidal base of awobanin (2×10^{-6} M), which was prepared from its chloride as before [11], was mixed with an aq. soln of flavocommelin (4×10^{-6} M). To several of these solns Mg acetate was added to give final Mg/awobanin mole ratios of 0–1.5 (0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.5). Each mixture was evaporated to dryness *in vacuo*, and the residue was re-dissolved in a small vol. of H_2O. Evaporation of the soln and re-soln of the residue in H_2O were repeated × 5. The blue aq. soln was then passed through a Sephadex LH 20 column (1.5 × 18 cm) using H_2O as eluant. A blue fraction of complex pigment most rapidly moving and easily separable from others was collected. The quantity of blue complex pigment formed was determined by measuring awobanin in the complex as described previously [5].

Commelinin and commelinin-like blue complex pigments. Crystalline specimens of commelinin and commelinin-like blue pigments synthesized previously [8] were used. The latter are the magnesium replaced blue complexes, i.e. Ni-, Cd-, Mn-, Zn- and Co-commelinins.

Absorption spectra of the blue pigments in buffer solns or in dilute HCl. Each blue pigment was dissolved in 0.05 M citrate–phosphate buffer (pH 2.4, 3.0, 4.0 and 5.0) or in dilute HCl (0.1% and 1%) in 100 mg/l. After standing for 10 min at room temp., absorption spectra were measured on a spectrophotometer (light path length of 3 mm) and then pH values of the solns were measured.

Dialysis of commelinin. Commelinin (*ca* 6–20 mg) was dissolved in a small amount of H_2O and the soln was dialysed for 24 hr through a cellophane membrane (Visking Cellulose Tubing) against 1 l of distilled H_2O under stirring at 5°. During the procedure the water was renewed × 8. The sample containing added Mg acetate (1.9 mg) was also dialysed under the same conditions. The blue dialysed soln was subjected to spectrophotometric determinations of Mg and awobanin as described previously [5].

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